5-1975

The Reproductive Cycle of Male Uinta Ground Squirrels and Its Relationship to Pineal N-Acetyltransferase and Monoamine Oxidase in the Testes, Hypothalamus and Pituitary

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THE REPRODUCTIVE CYCLE OF MALE UINTA GROUND SQUIRRELS AND ITS RELATIONSHIP TO PINEAL N-ACETYLTRANSFERASE AND MONOAMINE OXIDASE IN THE TESTES, HYPOTHALAMUS AND PITUITARY

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Biology

Approved:

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UTAH STATE UNIVERSITY
Logan, Utah

1975

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to my major professor, Dr. LeGrande C. Ellis, for his advice and guidance during this study and also for the funding of my research. I must also thank Dr. David F. Balph, Dr. Warren C. Foote, Dr. Ronald L. Urry for their assistance in this study. I am especially indebted to Dr. Michael Windham of the USU Mathematics Department for his personal assistance with the statistical analysis of the data. The technical help of Phyllis Taylor and Art Svejda was greatly appreciated.

I also wish to thank Dr. Thomas Oldfield for the assistance in trapping squirrels.

Finally, and most of all, I again thank my wife, Sylvia, for her typing of the preliminary forms of the manuscript and for her encouragement and prodding throughout my graduate career. May she now reap the long-delayed benefits of a gainfully employed husband.

Robert Alexander Palmer

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ABSTRACT

The Reproductive Cycle of Male Uinta Ground Squirrels
And Its Relationship to Pineal N-Acetyltransferase
And Monoamine Oxidase in the Testes,
Hypothalamus and Pituitary

by

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Utah State University, 1975

Major Professor: Dr. LeGrande C. Ellis
Department: Biology

A study was made of the reproductive cycle of male Uinta ground squirrels (Spermophilus armatus) during the activity seasons of 1973 and 1974 and during the aestivation/hibernation (A/H) period of 1973-1974. Physiological data collected were compared to changes in monoamine oxidase (MAO) activity of the testes, pituitary and hypothalamus and to pineal N-acetyltransferase activity.

Male Uinta ground squirrels began emerging from their burrows on April 12, 1973, and April 8, 1974, with average body weights of 302 g and 301 g, respectively. Body weight did not increase greatly during breeding, but rose rapidly to over a 500 g average prior to immergence into A/H. Body weight declined during A/H. Adrenal weight decreased in July, just prior to immergence into A/H and during A/H. An increase in adrenal weight occurred just prior to emergence from the burrows that was associated with and

thought to be partially responsible for the marked gonadal and reproductive development at this time.

Testicular development (as shown by testicular weight, length, width and seminiferous tubule diameter) was similar to data reported for this and other species of ground squirrels. Maximum gonadal development was observed at emergence that was followed by a regression to a basal state in June. A slight testicular recrudescence occurred during the preA/H period in July and during the A/H period, but maximum growth occurred during the period of proposed arousal in mid-March to the actual emergence time. Testicular histology of the germinal epithelium and interstitial cells followed a similar pattern of development except that the interstitial cells and accessory organs did not develop until just prior to emergence of the animals from their burrows.

Plasma testosterone levels and seminal vesicle weights were both highest at emergence and both decreased concomitant with testicular regression. There was no significant increase in either parameter during the preA/H testicular recrudescence.

Data presented here corroborated the hypothesis that FSH stimulates testicular MAO activity. First, total MAO activity changes were highly correlated ($r = 0.96$) with changes in testicular development that are most certainly under partial control of pituitary gonadotropins. Second, the proliferation of spermatogonia during preA/H and A/H periods, but the lack of development beyond the spermatogonia stage suggests FSH stimulation, but no effective LH or testosterone

stimulation. Third, the lack of interstitial cell development during preA/H and A/H suggests no effective LH stimulation. Fourth, the lack of an increase in plasma testosterone levels and seminal vesicle weights during preA/H and A/H periods also suggest no effective LH stimulation on the interstitial cell function. This increase in MAO activity with testicular development could protect the gonad from the deleterious effects of endogenous serotonin.

Pituitary and hypothalamic MAO followed the expected gonadotropin secretion pattern for testicular development found in the Uinta ground squirrel. These data suggest a functional role for MAO in controlling releasing factor and gonadotropin secretion by the hypothalamus and pituitary.

Diurnal variations in pineal N-acetyltransferase activity approached significance ($p < 0.07$) with 10:00 p.m. values greater than 10:00 a.m. values. A significant drop in pineal N-acetyltransferase activity in March just before arousal-emergence may facilitate testicular development at this time. High values at emergence may contribute to the rapid testicular regression immediately following emergence.

(114 pages)

INTRODUCTION

The Uinta ground squirrel, Spermophilus armatus,[1] enters aestivation/hibernation (A/H)[2] in the Logan Canyon area from mid-July through August and remains underground until emergence from their burrows in March or April. The squirrels are fully developed reproductively at emergence and breeding occurs soon after emergence from their burrows. Squirrels emerging around April 1 of 1959 and 1960 bred during the first two weeks of April (Balph and Stokes, 1963). Young are born after a 26-day gestation period and are seen above ground around 24 days after birth. Testes of reproductively active squirrels begin to regress in size before or shortly after emergence. The testes regress to a small fraction of peak-breeding size and ascend to an abdominal position by the end of May. Immediately prior to entering A/H, the testes increase slightly in size, partially descend to an inguinal position, and the males display some sexual behaviors (D. F. Balph, personal communication).

Three features of the squirrel's reproductive cycle differentiate them from some other seasonally breeding rodents. First, the squirrels

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[1]The common name and scientific name for each species of animal cited in this manuscript are given in the Appendix.

[2]The following abbreviations are used in this manuscript: monoamine oxidase--MAO; serotonin (5-hydroxytryptamine)--5-HT; 5-hydroxytryptophan--5-HTP; 5-hydroxyindole acetic acid--5-HIAA; norepinephrine--NE; catechol-O-methyl transferase--COMT; cyclic adenosine monophophate--c-AMP; N-acetyltransferase--NAT; hydroxyindole-O-methyl transferase--HIOMT; aestivation/hibernation--A/H; pre-aestivation/hibernation--preA/H; correlation coefficient--r; follicle stimulating hormone--FSH; luteinizing hormone--LH; and prolactin--PRL.

develop reproductively during A/H in complete darkness when they are presumably in a very inactive metabolic state. Second, regression of the testes occurs immediately after or just before emergence. Third, partial testicular recrudescence occurs just prior to the animals entering A/H in late July or August.

These changes in testicular development probably are related to changes in secretion of gonadotropin-releasing or inhibiting factors by the hypothalamus. Neurotransmitters, such as dopamine, serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE), probably are involved in the control of releasing factor secretion (Kamberi, Mical and Porter, 1970, 1971a, 1971b). Monoamine oxidase (MAO) is very important in regulating the levels of these brain amines (Neff and Yang, 1974) and, therefore, changes in MAO activity in the hypothalamus and pituitary may be very important in controlling gonadotropin release.

Another possible factor mediating gonadotropin release is the release of an antigonadal substance from the pineal gland in response to darkness. Reiter (1972a) has shown that short photoperiods (L:D, L:23) cause testicular regression in the golden hamster and that pinealectomy prevents the response. A dark-induced increase in pineal activity occurs in the laboratory rat as shown by the 15-30 fold increase in pineal N-acetyltransferase (NAT) activity, the rate-limiting step in melatonin synthesis (Klein et al., 1973). Changes in testicular development in the Uinta ground squirrel may also be related to changes in secretion of a pineal antigonadal agent.

Finally, testicular development may also be influenced by changes in testicular MAO activity, since this enzyme reportedly plays a

functional role in protecting the testes from endogenous 5-HT (Urry, Frehn and Ellis, 1974; Segal et al., 1975; Urry et al., 1975; Urry, personal communication).

The objectives of this research were threefold: a) to follow the reproductive activity of adult male Uinta ground squirrels through the active season and A/H period; b) to observe MAO changes in the adult male squirrel to ascertain if they are related to changes in sexual development during the reproductive cycle, and c) to observe pineal NAT activity and relate it to the reproductive activity of the male Uinta ground squirrel.

REVIEW OF LITERATURE

Ground Squirrels

Annual activity

Most members of the ground squirrel tribe of the family Sciuridae are hibernators in northern latitudes and high elevations and aestivators in lower latitudes and elevations. Uinta ground squirrels are an A/H species of Sciuridae. The activity season of this species begins from mid-March to mid-April when the squirrels emerge from their burrows, and lasts until late July and early August when the animals immerge into A/H (Balph and Stokes, 1963). Similar activity periods are shown by other ground squirrels that inhabit different latitudes, such as Franklin ground squirrel (Iverson and Turner, 1972), thirteen-lined ground squirrel (McCarley, 1966; Johnson, Foster and Coco, 1933), Richardson ground squirrel (Quanstrom, 1971), and golden mantled ground squirrel (McKeever, 1964). Most members of the group are diurnal, although the peaks in activity during the day are influenced by daily weather and season (McCarley, 1966).

Seasonal growth

The two main activities of the squirrels during the active season are to reproduce and to store energy for A/H. Body weight of emerging squirrels is low due to the utilization of energy stores during A/H. Body weight does not increase greatly during the breeding portion of the active season, but does increase rapidly thereafter (McKeever, 1964;

Mrosovsky and Fischer, 1970; Blake, 1972; Iverson and Turner, 1972; Knopf, 1973). Maximum body weight is attained just prior to A/H.

Adrenal weight changes for male golden mantled ground squirrels as reported by McKeever (1964) did not fluctuate greatly during the summer. A peak in adrenal weight occurred in April followed by a decline in May. The values plateaued through October then declined again through the fall--the preA/H period for these squirrels. Weights were low during the late A/H period--December through February.

Pituitary weights during the active season for golden mantled ground squirrels were fairly constant with only a slight increase during the season. A decline in pituitary weight occurred during October and November.

Reproduction

Adult ground squirrels of the family Sciuridae generally are in reproductive condition at the time of emergence from their burrows (Iverson and Turner, 1972; Sheppard, 1972; Michener, 1973; Slade, 1974), or come into reproductive condition shortly after emergence (Johnson, Foster and Coco, 1933; McKeever, 1964; McCarley, 1966). The Uinta ground squirrel has scrotal testes at emergence and the scrotum and foreskin are pigmented (Balph and Stokes, 1963). D. F. Balph (personal communication) has suggested that the testes of the squirrels reach a maximum size in mid-March, the earliest that squirrels have been reported to emerge, and at any later emergence, the males have a lower mean testicular size. Yearling male Uinta ground squirrels normally are not sexually developed at emergence and do not emerge

until after females have appeared above ground (Slade, 1974). However, Sheppard (1972) reported that all males of Richardson ground squirrels collected in early spring, including the yearling males (as determined by eye lens weight, epiphyseal closure, and molar wear), were in full reproductive condition.

Breeding occurs in April and May for most species of ground squirrels (Johnson, Foster and Coco, 1933; McKeever, 1964; McCarley, 1966; Iverson and Turner, 1972; Sheppard, 1972) and usually only one litter is born. McCarley (1966) reported that a few older females of the thirteen-lined ground squirrel had a second litter during the active season. The estimated conception dates were late May and early June.

The testes of most ground squirrels begin regressing soon after breeding and most squirrels are no longer sexually active by July (Johnson, Foster and Coco, 1933; McKeever, 1964; Iverson and Turner, 1972). McKeever (1964) found that a slight increase occurred in testes weights in late summer and fall during the preA/H period in the golden mantled ground squirrel. He also reported a continuing slow increase during A/H and a sharp increase at the end of A/H.

McKeever (1964) also reported on seminal vesicle weight change for golden mantled ground squirrels. Maximum weight was obtained in April, one month after peak testicular size. The gland atrophied rapidly and by June was the same size as juvenile seminal vesicles. The glands then remained at about this level until March when a rapid development occurred.

MAO

History and properties

Blascho (1972) attributes the discovery of MAO [monoamine: oxygen oxidoreductase (deaminating) EC. 1.4.3.4.] to the work of Hare in 1928 where she used tyramine as the substrate. Catecholamines were found to be substrates for the enzyme in 1937 (Blaschko, 1972). Bhagrat, Blaschko and Richter (1939) found high MAO activity in vertebrate liver, pancreas, heart, intestine, spleen, thyroid, kidney, brain, uterus and testes, but low activity in muscle. Since then a considerable interest in MAO has led to a greater understanding of the enzyme. Excellent reviews on MAO can be found in the works of Costa and Sandler (1972) and Neff and Yang (1974).

MAO functions in the oxidative deamination of monoamines to an aldehyde with the release of ammonia. The aldehydes are then converted either to tryptophols by alcohol dehydrogenase or to acids by aldehyde dehydrogenase (E.C. 1.2.1.3.). Most intercellular MAO activity occurs on the outer membrane of mitochondria (Kroon and Veldstra, 1972; Masters, 1972). Whether MAO enzyme is found in the cytoplasm or other portions of the cell is under debate (Blaschko, 1972). Cytoplasmic MAO may be newly synthesized enzyme on the endoplasmic reticulum, enzyme detached from the outer mitochondrial membrane or fragments of outer mitochondrial membrane not precipitated with the intact mitochondria. Other amine oxidases have been found. Plasma MAO (also called benxylamine oxidase), diamine oxidase and connective tissue MAO also occur and are similar to each other in that they require

copper and contain pyridoxal phosphate (Blaschko, 1972). Brain MAO has also been regarded as requiring copper for activity (Barbato and Abood, 1963; Nara and Yasunobu, 1966). However recent reports indicate that copper can be removed without the loss of activity (Nagatsu et al., 1972). In contrast to the other amine oxidases mentioned, brain MAO contains FAD rather than pyridoxal phosphate.

Multiple forms of mitochondrial MAO have been demonstrated by several researchers (Nagatsu et al., 1972; Yang, Goridis and Neff, 1972; Tipton, Alouslay, and Garrett, 1973; Youdim, 1973; Neff and Yang, 1974). Nagatsu et al. (1972) compared the two components of bovine brain mitochondrial MAO and found that one component was very active with tyramine as a substrate while the other component reacted strongly with normetanephrine. Yang, Goridis and Neff (1972) found the MAO of pineal mitochondria to have a greater activity with tyrosine than did superior cervical ganglion MAO. Youdim (1973) used polyacrylamide-gel electrophoresis to separate rat brain MAO into at least four bands and rat liver MAO into five bands, all with enzymatic activity. These different forms had, in preliminary studies, different properties. Little is known of the possible nature of these multiple forms. Tipton, Alouslay and Garrett (1973) suggest that the forms of the enzyme are the result of lipid membrane materials associated with the enzyme. When MAO preparations were treated with sodium perchlorate, which removes lipid material, and then subjected to gel filtration, the multiple band electrophoretic pattern is lost and only one band remains. No difference could be detected in activity between the treated and

untreated enzyme preparations. The effects of an MAO inhibitor, clorgyline, with different substrates on treated and untreated enzyme preparations showed that treatment with perchlorate abolished the different dose-response curves obtained with untreated enzyme preparations. However, both Tipton, Alouslay and Garrett (1973) and Youdim (1973) point out that the different enzyme forms may be only an artifact of the preparation technique and that these forms may not exist *in vivo*.

Various types of MAO have been observed on the basis of their response to various substrates and inhibitors. Johnson (1968) suggested that two types of enzyme must occur because of the response of brain MAO to clorgyline. Neff and Yang (1974) reported on the probable substrates and inhibitors that affect these two types. One type, type-A, had preferred substrates of 5-HT, NE and normetanephrine, while type-B MAO was more specific for benzylamine and beta-phenylethylamine. Dopamine, tyramine and tryptamine were accepted as substrates by both types. Clorgyline preferentially inhibited type-A MAO while pargyline, iproniazid, phenylzine and nialamine all inhibited both types. Kroon and Veldstra (1972) also reported two enzyme types with different substrate specificities: 5-HT and dopamine were specific substrates for one type while NE and kynuramine were preferred substrates for the other isoenzyme.

Monoamine oxidase activity of any one tissue changes during the life of the animal. Baker, Hoff and Smith (1974) studied the maturation of MAO in brain tissue of mice and found that each region had an

individual pattern of development. Robinson et al. (1972) found that human hind brain MAO decreased from age 25 to 35 years in humans, then increased from 35 years to older than 70 years. Plasma and platelet MAO's followed the same pattern, although most plasma MAO increases occurred from age 55 to over 70 years. These hind brain MAO changes were negatively correlated to NE ($r = -0.54$, $p < 0.005$) and positively correlated to 5-hydroxyindole acetic acid (5-HIAA, $r = +0.55$, $p < 0.001$). Vaccari, Cugurra and Maura (1972) compared MAO activity at 1, 3, 9 and 18 days to adult activity levels in rat liver, brain, spleen and in smooth muscle of the stomach. Values were significantly lower than adult levels at one and three days in the brain, spleen and stomach samples while the levels were greater in the liver for these two days. The activity levels were lower on days 9 and 18, then increased generally thereafter. Vaccari et al. (1972) reported that all tissues reached adult levels by day 18. Gripois and Roffi (1972) reported similar findings in that adult liver and kidney MAO levels were reached at day 18. Pentilla and Kormano (1968) studied MAO activity in rat testes during prenatal and postnatal periods. In 19-day-old embryos and in newborn rats the Leydig cell and sex cord cell activity was higher than adult levels as shown by histochemical analysis. The values were about the same as adult levels by day 35. Quantitative assays revealed a high value at day 19 of embryonic life but the highest value was observed on day 1. The activity declined to day 35, then the activity increased to adult levels. Similar findings were reported by Ellis et al. (1972). Additionally, Ellis et al. (1972) found a decline in activity with aging of the animal.

Catecholamines and 5-HT in the brain

Dopamine, NE and 5-HT are neurotransmitters found in the central nervous system that are metabolized by MAO. Excellent reviews of catecholamine and 5-HT metabolism can be found in the work of Axelrod (1965), Costa and Meek (1974), and Snyder (1972). Dopamine and NE are synthesized from tyrosine, a dietary amino acid, by the enzyme tyrosine hydroxylase and DOPA-decarboxylase. These catecholamines are stored in synaptic vesicles and are released by exocytosis. Inactivation occurs by re-uptake of the transmitter from the synaptic cleft by the presynaptic neuron, by O-methylation due to the enzyme, catechole-O-methyl transferase (COMT) in the synaptic cleft, or by oxidative deamination by MAO.

Serotonin is synthesized from tryptophan, another dietary amino acid, by tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase. It is stored in vesicles and is probably released by exocytosis. Inactivation most likely occurs in a similar manner to catecholamines, by re-uptake into the presynaptic neuron or by MAO. COMT does not participate in 5-HT inactivation.

Dopamine, NE and 5-HT all play important roles in the control of reproduction (as well as other physiological processes) by the central nervous system. Changes in the levels of these compounds and in the activity of their respective neurons occur on a daily and seasonal basis (Hery, Rouer and Glowinski, 1972; Feist and Galster, 1974). Kamberi, Mical and Porter (1970, 1971a, 1971b) worked with release of FSH, LH and prolactin (PRL) in rats due to catecholamines. Dopamine administered into the third ventricle caused the release of FSH and LH

and inhibited PRL release while NE had no effect on either, except at high concentrations. Intraventricular injection of 5-HT caused a significant drop in the plasma levels of LH (Kamberi, Mical and Porter, 1970). Craven and McDonald (1973), however, failed to show an effect of either dopamine or NE in causing ovulation in reserpine treated estrous rats. Dopamine inhibited ovulation in normal rats (Craven and McDonald, 1973). These transmitters are thought to control reproduction by mediating the release of gonadotropin releasing factors (Muller et al., 1972).

Ladosky and Gaziri (1970) reported different changes in rat brain 5-HT between males and females at the time of differentiation of the hypothalamus. Serotonin was high on day 12 in normal females and in males castrated on day 1, while low values on day 12 were obtained in males and females injected with androgens on day 1. In later work, Ladosky and Noronha (1974) demonstrated an inhibitory role of 5-HT on ovulation. In contrast, Wilson and McDonald (1974) concluded that there was no inhibitory effect of 5-HT on the cycle ovulatory surge of LH. Serotonin is involved with the release of PRL (Kamberi, Mical and Porter, 1971c; Kordon et al., 1973; Calgarus and Taleisnik, 1974). Kamberi, Mical and Porter (1971c) reported that 5-HT stimulated prolactin release, but inhibited FSH release.

MAO and brain function

MAO is found on the outer layer of the mitochondri in the dopaminergic, adrenergic and serotonergic neurons (Schnaitman, Erwin and Greenawalt, 1967). The main function for MAO in this tissue is to

metabolize the neurotransmitters that are free in the cytoplasm of the neuron and thereby control the amount of transmitter available (Kopin, 1964; Neff and Goridis, 1972; Neff and Yang, 1974). MAO does not metabolize transmitters released by exocytosis unless they are taken up by the neuron into the neuroplasm but not bound into synaptic vesicles (Smith, 1966). MAO inhibitors that block the degradation of monoamines result in an increase in monoamine levels until de novo synthesis of MAO reestablishes normal monoamine levels (Glowinski et al., 1972; Neff and Yang, 1974).

Activity levels of MAO in the brain are not static but change with different physiological states. Maura et al. (1974) subjected rats to acute and chronic stresses in the form of auditory stimulation, flashing lights and cage oscillation. Acute stress did not change MAO activities, but chronic stress reduced MAO activity in the heart, brain and liver. This may be due to the inhibitory effect of adrenocorticotropic hormones on MAO activity as reflected by the work of Petrovic and Janic (1974). Gaziri and Lodosky (1973) reported changes in MAO activity during sexual differentiation of the rat hypothalamus. On day 12, when female 5-HT levels were greater than male levels, MAO activity was greater in the anterior hypothalamus in the males than in the females. No difference could be found in the posterior hypothalamus on any day. These workers concluded that changes in MAO activity may play an important role in the sexual differentiation of the rat hypothalamus.

MAO activity also changes during the estrous cycle in rats. Zolovick et al. (1966) and Kamberi and Kobayashi (1970) both found

higher MAO levels in proestrus and estrus of the estrous cycle while Holzbauer and Youdim (1973) found highest activity during proestrus and diestrus, with the lowest activity on the day of estrus.

MAO and testicular function

Another suggested function for MAO is the protection of the body from toxic monoamines that may be produced in the body or that may be ingested in the diet. These toxic amines may have pharmacologic functions such as constriction of vascular beds or cause the release of other catecholamines (Neff and Goridis, 1972; Ng et al., 1972; Everett, 1974). MAO in the testis may have such a role in protecting the testes from the deleterious effects of 5-HT that is found in the testis in varying amounts depending on the age and physiological state of the animal. Zieher et al. (1971) reported decreasing amounts in rats from birth (highest 4.39 µg/g tissue) to maturity (lowest 0.10 µg/g).

Exogenously administered 5-HT was shown to accumulate in different amounts in different reproductive tissues of male rats in a study by Hodgen and Gawienowski (1972). A moderate amount of 5-HT was found in the testis parenchyma and tunica albuginea while the greatest amount was observed in the pampiniform plexus and testicular artery. Less 5-HT accumulated in the corpus and cauda epididymis and penis. In contrast, most exogenously administered 5-hydroxytryptophan accumulated in the testicular parenchyma while least was found in the pampiniform plexus and testicular artery. Overall, most 5-HT accumulated in high vascular areas, possibly because of high sympathetic innervation, while the precursor of 5-HT, 5-hydroxytryptophan, accumulated in parenchyma.

Kormano and Pentilla (1968) found no significant increase in testicular 5-HT content after exogenous administration, but did report a constriction of the testicular artery. In contrast to the findings of Hodgen and Gawienowski (1972), however, three times as much 5-HT accumulated in the epididymis of adult rats.

Boccabella, Salgado and Alger (1962) studied the effects of exogenous 5-HT (10 mg/kg twice daily) on the testes. Testicular weight decreased significantly and the seminiferous tubules degenerated so that only Sertoli cells and spermatogonia remained after 15 days of treatment. Androgen production apparently decreased since the seminal vesicle weights decreased significantly. However, O'Steen (1963) found no effect of a single 5-HT injection of 6.25 mg/kg on testicular weights, although some seminiferous tubules showed signs of degeneration. When Boccabella, Salgado and Alger (1962) administered 5-HT in conjunction with apresoline, a vasodilator, the harmful effects of 5-HT on testicular weight and histology were blocked. Joffre and Joffre (1973) demonstrated substantial seasonal changes in testicular blood flow of several seasonal breeding mammals. Marley (1964) demonstrated that 5-HT blocked embryo implantation in the uterus when a dose of 20-40 mg/kg was given subcutaneously at the time of implantation and these same effects could be produced by vasoconstriction of the uterus. One antigonadal action of 5-HT may, therefore, be likely due to a vasoconstrictor action.

Serotonin may also have a direct action on androgen synthesis by the testis. Ellis (1969, 1972) demonstrated that in rats 5-HT blocked

androgen synthesis *in vitro* and increased testosterone metabolism. Liu and Kinson (1973) reported reduced levels of testosterone in mixed venous blood after peripheral administration in the rat. Mieno et al. (1973) could not show an *in vivo* effect of 5-HT (0.5 mg/kg) on canine testicular secretion of androgens. However, most samples of Mieno's et al. (1973) study had testosterone levels below the sensitivity of his assay and, therefore, may not accurately show changes in plasma testosterone levels.

Protection from endogenous 5-HT levels could be provided by testicular MAO. MAO is found in the testes (Bhagrat, Blaschko and Richter, 1939; Penttila and Kormano, 1968; Urry, Frehn and Ellis, 1974). Penttila and Kormano (1968) localized the activity by histofluorescence in the interstitial cells and in the basal cell layers of the seminiferous tubules. According to these researchers, the MAO distribution is the same as the distribution of exogenously administered monoamines.

Penttila and Kormano (1968) also reported changes in testicular MAO with age in laboratory rats. Nineteen-day-old fetuses had a moderate level of activity while newborn rats had the highest levels. A decline followed until about day 35, then an increase to adult levels. Ellis et al. (1972) found a similar increase in rat testicular MAO activity at 105 days from a low at 41 and 57 days, then a similar low value at 365 days. MAO changes followed the development of the rat testis as shown by androgen synthesis (Ellis et al., 1972).

Seasonal changes in MAO activity occur (Petrovic et al., 1974; Urry et al., 1975). Petrovic et al. (1974) found highest hypothalamic

and liver MAO levels in the European suslik in September with lowest levels in February and only slightly higher levels in April. Urry et al. (1975) reported high MAO activities in the house sparrow during the breeding season (March through June) and low activities during the non-breeding period (August through January). Urry et al. (1975) also reported in preliminary data that MAO activity in the testes of Uinta ground squirrels was highest in April at emergence and lowest in May toward the end of the breeding season. In both animals the MAO activity appeared to closely follow the development of the testis.

Testicular MAO activity has been suggested to be under the control of FSH from the pituitary. Urry, Frehn and Ellis (1974) demonstrated that injections of FSH, but not LH, PRL, or a combination of all three could significantly increase testicular MAO. FSH also increased MAO activity in tissue culture. Frehn et al. (1972) reported that light inhibited testicular MAO of individually caged Uinta ground squirrels and crowding lowered testicular activity in squirrels exposed to total darkness.

Pineal Gland

Development and anatomy

The pineal gland or epiphysis cerebri has been intensively studied since Lerner and his co-workers characterized melatonin as an emphibian skin lightening agent in the late 1950's and early 1960's (Lerner et al., 1958). The last 15 years has seen a tremendous expansion of our knowledge of the pineal and the publication of a considerable number

of papers. Excellent reviews have been written and the reader is referred to them for more detailed accounts of pineal function (Kappers and Schade, 1964; Wurtman, Axelrod and Kelly, 1968; Reiter, 1973a, 1973b; Axelrod, 1974; Quay, 1974).

The pineal gland in mammals develops from an evagination of the roof of the diencephalon just anterior to the posterior commissure (Quay, 1974). Nervous connection with the brain is lost early in life and only aberrant commissural fibers pass through the pineal from the central nervous system (Mollgaard and Moller, 1973). The pineal is innervated by postganglionic sympathetic fibers that have their cell bodies in the superior cervical ganglia. These nerves reach the pineal via the nervi conarii (Mollgaard and Moller, 1973). Blood is carried to the pineal via small arterioles branching from the posterior choriod arteries. It leaves the pineal via small veins that drain into the greater cerebral vein of Galen and then enters the systemic circulation via the internal jugular vein (Tamaki et al., 1973). No portal system has been demonstrated between the pineal and other brain regions, but it has been suggested that a communication may exist by reversed blood flow through the greater cerebral vein and the choroid plexus (Quay, 1974). However, this has not been demonstrated to occur in vivo.

Embryonically, pineal cells are derived from neural ectoderm and mesoderm (Quay, 1974). The pinealocytes, the main cell type of pineal parenchyma, come from the neuroectoderm while the connective and circulatory tissues result from mesodermal investments. Pinealocytes are club shaped cells with the base of the cell endings in a perivascular

space surrounding a capillary (Pevet and Saboureau, 1973; Quay, 1974). Secretory granules occur in two main sizes, small clear vesicles (350-450 Å) and dense-cored vesicles (400-1500 Å), both produced by the Golgi apparatus. These vesicles appear to migrate toward the terminal process (Pevet and Saboureau, 1973). Sympathetic neurons appear to terminate on the pinealocytes (Quay, 1974).

Melatonin synthesis

Axelrod (1974) reviewed the synthesis of melatonin in the pineal, presumably by the pinealocytes. Dietary tryptophan is taken up by the pineal and converted to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase. The 5-HTP is converted by l-aromatic amino acid decarboxylase (may be the same as DOPA decarboxylase) into 5-HT. The 5-HT can be either converted to N-acetylserotonin by the enzyme NAT or can be metabolized by MAO to 5-HIAA (see MAO review). The N-acetylserotonin formation is considered by many to be the rate-limiting step in melatonin synthesis (Klein, Weller and Moore, 1971). N-acetylserotonin is converted into melatonin by the enzyme hydroxyindole-O-methyl transferase (HIOMT). HIOMT is an enzyme peculiar to the pineal gland (Axelrod, 1974), but is also located in the rat retina and Harderian gland (Cardinali, Larin and Wurtman, 1972). However, no melatonin synthesis has been demonstrated in either the retina or Harderian gland as yet (Quay, 1974).

Photoperiod control

The various steps in melatonin synthesis do not occur at a homogeneous rate throughout the day, but have definite circadian variations. A circadian rhythm in pineal 5-HT was first demonstrated by Quay (1963). He found a maximum concentration of 5-HT at the midpoint of the light period and a rapid fall in concentration immediately after the beginning of the dark period. Leaving the lights on at the beginning of the dark period prevented the decline in 5-HT while turning the lights on during the dark period when the 5-HT concentration was low resulted in an increase in 5-HT. This pineal rhythm is now considered to be an endogenous rhythm which is entrained by photic stimulation, but is free running in continuous darkness (Illnerova, 1972). Photoresponsive rhythms occur with several other steps in melatonin synthesis: namely NAT activity (Klein and Weller, 1970), HIOMT activity (Illnerova, 1972), NE release (Brownstein and Axelrod, 1974), pineal sensitivity to isoproterenol, a beta-adrenergic stimulator (Romero and Axelrod, 1974) and in adenosine 3', 5'-monophosphate c-AMP (Klein, Berg and Weller, 1970). A circadian rhythm of melotonin secretion in man has been found with a peak between 11:00 p.m. and 7:00 a.m. compared to 7:00 a.m. to 3:00 p.m. and 3:00 p.m. to 11:00 p.m. levels (Lynch et al., 1975).

Quay (1974) completed an excellent review of the changes occurring in the pineal with respect to these rhythms. At the beginning of the light cycle, the amount of NE falls, possibly because of its release from storage sites (Brownstein and Axelrod, 1974). This release stimulates the conversion of tryptophan to 5-HTP by c-AMP stimulation

of tryptophan hydroxylase activity. This results in an increased synthesis of 5-HT which is then divided between two pools. Some is retained by the pineal parenchyma cells and converted to melatonin while the rest is taken up by sympathetic nerve terminals. The latter is either stored in the terminal vesicles or metabolized by neuronal MAO to 5-HIAA, which contributes to an increase in 5-HIAA during the day. Daytime c-AMP levels increase to nearly six times those of the dark period (Quay, 1974).

When the animal enters the dark phase of the cycle, the first event is a rapid decline in 5-HIAA, presumably because of a release of 5-HT from the nerve terminals and, therefore, a reduction in the amount of 5-HT available for MAO metabolism (Quay, 1974). The released 5-HT is probably picked up by the pinealocytes again where it can be converted to melatonin. At the same time, the activity of the nerves is greatly increased as measured by the reduced turnover time of NE (Brownstein and Axelrod, 1974). This increase in NE stimulates adenyl cyclase on pinealocyte membrane to increase c-AMP. This then activates NAT, melatonin synthesis (Klein et al., 1973). Romero and Axelrod (1974) have shown that in the early dark phase the pineal is more sensitive to beta-adrenergic agonists such as isoproterenol than it is in the early light phase. N-acetylserotonin is then converted into melatonin by HIOMT resulting in a dramatic rise in melatonin soon after the dark phase begins.

If at the beginning of the dark phase the lights are left on, the changes in enzyme activity do not occur. There is no drop in 5-HT

concentration (Quay, 1974), there is no increase in NE turnover (Brownstein and Axelrod, 1973) and there is no stimulation of NAT activity (Binkley, Klein and Weller, 1973). However, there appears to be a refractory period to darkness since darkness is effective in stimulating NAT activity only during the expected time of darkness and is ineffective at other times. If the lights are turned on during darkness, a sudden drop in NAT activity occurs (Deguchi and Axelrod, 1972a) and 5-HT accumulates (Quay, 1974). Light, then, inhibits the stimulation of the pineal by blocking the activity of the post-ganglionic sympathetic nerves terminating in the pineal (Axelrod, 1974).

In mammals, photostimulation is first received by the eyes, as has been shown by complete darkness and enucleation experiments. Animals placed in complete darkness continue to have rhythms in pineal melatonin production, but the cycles tended to become free running and asynchronous with respect to other individuals after a few days (Klein and Weller, 1970). The same phenomenon occurs in enucleated animals even when kept in continuous light (Klein, Weller and Moore, 1971). The eyes are necessary for perception of environmental light conditions. Photic stimulation reaches the pineal through the following pathway: retina, inferior accessory optic tract, medial forbrain bundle, medial terminal nucleus of the accessory optic system, preganglionic sympathetic tract in the spinal cord, superior cervical ganglia, postganglionic sympathetic fibers, and finally parenchymal cells (Axelrod, 1974). Interruption of this pathway by enucleation, superior cervical ganglionectomy or by decentralization of superior cervical ganglia blocks the

pineal rhythm. However, electrical stimulation of the superior cervical ganglia results in a three-fold increase in NAT activity (Deguchi and Axelrod, 1973).

Pineal and reproduction

Animals in natural environments have evolved breeding patterns to insure that offspring are born at the most advantageous period of the year for their growth and survival. Consequently, the mating season usually occurs at such a time as to allow the young to be born at the most appropriate time of year (Farner and Follett, 1966). Although many cues may be used to time the reproductive cycle, photoperiod changes are the most consistent cues available (Farner and Follet, 1966). It has been known for many years that animals respond reproductively to changes in photoperiods. Many mammals with relatively short gestation periods respond reproductively to lengthening photoperiods. Examples are rabbits, rats, hamster, ferrets and mink. The horse, which has a gestation period of almost a year, also responds to long photoperiods. However, intermediate gestation lengths would require mating in the fall during short photoperiods. Sheep and goats with gestational periods of around half a year or less respond reproductively to short-day photoperiod. Marshall and Swan (1971) in a study of growth patterns of blind and normal human children found the patterns to be six months out of phase between the northern and southern hemispheres. In the northern hemisphere, the period of maximum growth for normal children occurred between January and June while the blind and partially blind children's growth maximums occurred throughout

the year. Duby and Travis (1972) reported that placing mink in a gradually longer photoperiod induced a change to summer pelage and also caused the spring reproductive development of the mink to occur earlier.

It has been known for some time that the pineal gland was involved with reproductive responses to photoperiod. Clausen and Poris in 1937 found that pinealectomy of the American chameleon resulted in increased testicular size. However, it was not till after the demonstration of melatonin synthesis in the pineal that the antigonadal nature of the pineal was fully realized.

Effects of the pineal on female reproductive physiology have also been demonstrated. Vaughn et al. (1972) and Reiter, Vaughn and Vaughn (1972) have both shown that a single dose of melatonin will inhibit compensatory ovarian hypertrophy (the growth of the remaining ovary when one ovary is removed surgically), whereas 5-HT was found to be ineffective. Melatonin also prevents ovulation in normally cycling rats if given on the afternoon of proestrus (Ying and Greep, 1973). The results of pinealoectomy on ovarian function are equivocal. While DeFronzo and Roth (1971) reported that many researchers found a growth of the ovaries after pineal removal, they themselves were unable to demonstrate this. This difference may be due to the time after pinealectomy that the ovaries were weighed; two to four weeks in the case of the other researchers while DeFronzo and Roth waited for six weeks. Reiter (1973a) reported that blinded rats produced less milk than normal rats and the effect was pineal-related. Blinding or short light exposure resulted in delayed vaginal opening while continuous light exposure hastened puberty in young rats (Reiter, 1973c).

The pineal gland and testicular development of male golden hamsters have been extensively studied by Reiter and his associates (Reiter, 1972a, 1972b; Reiter et al., 1974). Placing an adult male hamster in a short photoperiod (L:D, 1:23) for ten weeks results in atrophy of the testes. However, pinealectomy before exposure to L:D, 1:23 prevents the regression of the testes. Re-exposure of L:D, 1:23 hamsters to L:D, 14:10 after testicular regression results in recrudescence of the testes. Exposure of male hamsters to L:D, 1:23 for 30 weeks or more results in testicular recrudescence, even in the dark. Seibel and Schweisthal (1973) reported that blinding of male and female hamsters resulted in gonadal and accessory organ atrophy that could be prevented by enucleation. Interruption of the sympathetic nervous connection to the pineal by superior cervical ganglionectomy also resulted in blocking the antigonadel effect of L:D, 1:23 (Reiter, 1972b). Hoffmann (1973) reported similar findings for different photoperiod exposures and melatonin administration to Djungarian hamster. Exposure to a long photoperiod in January increased testes size compared to summer weights, while injection of melatonin into animals exposed to a long photoperiod delayed testicular development.

Somewhat similar, but not as drastic, results have been found in the rat, although all results are not unequivocal. Reiter (1968) reported that puberty was delayed in male rats subjected to limited light early in life while in young pinealectomized males puberty occurred at the normal time. Reiter (1968) also reported that blinding rats at age 25 diminished testicular weights and accessory organ weights

for over 150 days. Debeljuk et al. (1971) reported that melatonin administration caused testicular regression in rats. However, Liu and Kinson (1973) could not demonstrate an effect of melatonin on testicular weights after peripheral administration. Reiter et al. (1974) and Hoffman (1974) have even demonstrated that melatonin administration blocked the effect of L:D, 1:23 in causing testicular regression in golden and Djungarian hamsters, respectively.

Melatonin has been shown to inhibit testosterone production both in vitro (Ellis, 1969, 1972) and in vivo (Liu and Kinson, 1973). Blinding of 12-week-old rats resulted in reduced testosterone production 12 weeks later while pinealectomy, although it transiently reduced androgen levels, had no effect at 12 weeks (Liu and Kinson, 1973).

Melatonin is not the only antigonadal agent that has been isolated from the pineal gland. A watersoluble extract with antigonadal and antidiuretic properties had been found which is suspected of being arginine vasotocin (Konig and Meyer, 1971; Mattews, Benson and Rodin, 1971; Pavel, 1971; Benson, Mattews and Rodin, 1972; Orts and Benson, 1973; Pavel et al., 1974; Vaughan, Vaughan and Klein, 1974). A discussion of this pineal antigonadotropin can be found in Quay (1974).

It is thought that the pineal exerts its antigonadal effects through the action of its products on the hypothalamus or on the gonadal organs directly. The former is more likely in that decreasing photoperiods stimulate pineal activity causing synthesis and release of an antigonadal agent into the blood where it could be carried to the hypothalamus and alter the neural activity there. Anton-Tay et al. (1968) reported that intraperitoneal injection of melatonin

increased whole-brain, midbrain, and hypothalmic 5-HT content. Melatonin also has been shown to increase NE and dopamine in the rat brain after intravenous or intracisternal injection (Wendel, Waterbury and Pearce, 1974). All three of these amines have been implicated in gonadotropin releasing hormone release (see MAO section also). Oral administration of melatonin resulted in a rapid rise in growth hormone levels in human blood (Smythe and Lazaras, 1974). Melatonin injected into the third ventricle resulted in increased PRL levels in the blood of rats, but a decreased FSH level (Kamberi, Mical and Porter, 1971c). Increased PRL release after melatonin administration was also found by Lu and Meites (1973). In contrast, Ronnekleiv, Krolich and McCann (1973) reported that pinealectomy removed the early morning surge of PRL found in normal adult male rats. The normal surge occurred at a time when melatonin was low and not in the middle of the dark-phase when melatonin release was high. Moszkowska et al. (1973) reported finding that a certain fraction of gel filtration of pineal homogenate, previously shown to reduce pituitary FSH secretion, also caused an increase in FSH releasing factor and LH releasing factor in the hypothalamus. Their interpretation is that the secretion of releasing factors by the hypothalamus was blocked thus reducing FSH and LH release from the pituitary.

Seasonal changes

Studies on the involvement of the pineal in annual reproductive rhythms are not numerous. Already mentioned was the study on the Djungarian hamster by Hoffmann (1973). Reiter has studied the golden

hamster extensively, especially in terms of its seasonal reproductive cycle and its relation to photoperiod and pineal function (Reiter, 1973c). In its natural environment, the hamster hibernates during the winter, emerges in the spring in full reproductive capacity, breeds during the early spring and summer, then experiences a gonadal regression prior to entering A/H (Reiter, 1973a). Even in captivity in longer photoperiods and warmer temperatures the male hamster experiences a decline in reproductive capacity during the winter (Reiter, 1973c). Pinealectomy blocks the response of the gonadal axis to short photoperiods and the animals remain reproductively active during the winter (Reiter, 1973c). This demonstrates that the pineal is responsible for the photoperiodic control of the reproductive rhythm in the golden hamster.

In another rodent species, the whitefooted mouse, Lynch (1973) demonstrated an effect of photoperiod on fall molting and reproduction, but temperature had no effect on these parameters. Mice were kept in warm (26 C) and cold (5 C) chambers and in either L:D, 16:8 or L:D, 9:15 photoperiods. The short photoperiod induced a fall molting pattern and testicular regression in either warm or cold conditions, but no change in these parameters was found with maintenance in long photoperiods.

Ellis and Balph (1975) reported changes in HIOMT activity in the Uinta ground squirrel for different age groups at three different periods of the activity season: breeding period, postbreeding period, and preA/H period. In juvenile male squirrels HIOMT activity was high during preA/H. Yearling males had significantly lower activity during

the breeding period than the juvenile preA/H period values and showed a marked but non-significant increase during the activity season. Three-year-old squirrels had significantly lower HIOMT activities during the breeding period than the preceding fall (2-year-old activities during preA/H) and also had lower activities during the breeding period than the postbreeding period. The juveniles, one-year-old and three-year-old squirrels all had increasing HIOMT activities during the active season. However, these changes may not be due to changing photoperiod effects on the pineal of the squirrel. Frehn (1972) was unable to demonstrate a decrease on the pineal HIOMT activity in squirrels subjected to L:D 14:10 schedule at 70 degrees for 21 days in late September.

Barfuss and Ellis (1971) in a seasonal study of HIOMT activity and reproduction in the house sparrow found an inverse relationship between HIOMT and testes weight, but concluded that the pineal gland did not appear to be the driving factor behind the reproductive cycles. However, HIOMT activity did increase in birds caught in April and placed for 20 days in darkness compared to a similar group maintained in red light for the same time period. The testes of the birds in continuous darkness were also smaller than the red-light treated group. These workers showed that the house sparrow pineal is responsive to light, but the pineal does not appear to mediate the seasonal gonadal rhythm.

METHODS

Collecting

Uinta ground squirrels were collected in the vicinity of the Utah State University Forestry Camp (1909 m elevation), Franklin Basin (2035 m), and in the vicinity of Tony Grove Lake (2,438 m). National live traps were used with peanut butter and rolled oats as bait. Traps were set out before sunrise and left until an hour after sunrise. Adult males were collected while all other animals were released. The traps were reset and left for another hour. The traps were then collected and the adult males were retained and processed. In the early spring, all males with scrotal testes were considered to be adults. After this, all males were considered adult age if their weight and size exceeded that of the females. This meant that in the early summer, body weights were 300 g or more, while in late summer the weights were approximately 500 g. Trapping began as soon as possible after the squirrels emerged from their burrows in the spring and was repeated in approximately two-week intervals. During late May and June the trapping interval was greater because of less dramatic changes in reproductive states.

Experiments

The squirrels were studied in three experiments. First, adult male squirrels were collected at approximately two week intervals from

first emergence to entrance into A/H for data during the spring-summer season of 1973 and 1974. Second, 20 squirrels were trapped in the vicinity of Tony Grove Lake during August, 1973, for use in the A/H experiment. These squirrels were maintained in an environmental chamber in complete darkness at 4 C. Five squirrels were sacrificed in one month intervals beginning on December 14, 1973. Third, 14 adult male squirrels were collected on August, 1974, near Tony Grove Lake for use in the pineal photoperiod experiment described later. All squirrels maintained in the lab were fed Purina Lab Chow and water ad libitium supplemented with lettuce greens.

Processing

Squirrels were sacrificed in the field by cervical dislocation. A blood sample was immediately taken by cardiac puncture. The animal was then weighed to the nearest gram. The pineal, pituitary, anterior and medial hypothalamus and testes were dissected free and placed in a vial of 0.33 M buffer, pH 7.4 (0.72 M phosphate; NaCl, 1.125 g; KCl, 0.0575 g; anhydrous $MgSO_4$, 0.0477 g; anhydrous K_2HPO_4, 1.8444 g; $NaH_2PO_4 \cdot H_2O$, 0.2624 g; in 100 ml water). These tissues were immediately frozen on dry ice and kept frozen (-20 C) until assayed. One cauda epididymis of each male was sectioned and blotted on a glass slide for microscopic determination of sperm content and scored as sperm present or absent. The squirrel carcasses were placed on ice until return to the laboratory. There the adrenal glands, seminal vesicle and prostate glands were removed and weighed. The hypothalamic tissues were sectioned following the methods of Glowinski and Iverson (1966) into anterior and

medial portions. The length and width of the testes were recorded with the use of a divider and millimeter rule before being frozen.

Squirrels used in the photoperiod and A/H experiments were handled in the same manner except that the processing occurred in the laboratory. Small portions of testes of A/H squirrels were placed, at the time of sacrifice, in Bouin's fixative for later histological examination. Portions of testes from squirrels caught in the field were placed in Bouin's fixative while still frozen at the time of assay. Histological sections of the imbedded tissue were stained with hemotoxylin and eosin. The average diameter of the seminiferous tubules (five tubules cut in cross-section) was measured with a calibrated ocular micrometer. Sperm in the tubules was noted as being either present or absent.

Pineal NAT Activity

The activity of the pineal enzyme NAT was assayed by following the methods of Klein, Weller and Moore (1971). Pineals were homogenized in 20 ml of solution 1 mM in [^{14}C]-5-HT (Schwarz/Mann, 47 mC/mmole), 2 mM in acetyl CoA, and 0.1 M phosphate buffer, pH 6.8. After incubation for 10 min at 37 C, the radiolabeled N-acetylserotonin and melatonin were isolated by thin-layer chromatography and counted in a liquid scintillation counter (Packard Instrument Co.).

MAO Activity

MAO activity was assayed in the anterior and medial hypothalamus, pituitary and testes following the second procedure of Urry, Jaussi and Ellis (1972). Two mg homogenized tissue were incubated in 60 ml

of phosphate buffered solution containing 0.5 ml 5-HT[^{14}C] (0.65 mM). Radiolabeled 5-HIAA formed was extracted with ethyl acetate, purified by exchange over 0.3 N HCl, and counted in a liquid scintillation counter (Packard Instrument Co.).

Radioimmunoassay for Testosterone

Blood collected in the field was kept on ice until reaching the laboratory. Blood collected in the laboratory was placed in the refrigerator. After clotting, the blood was centrifuged and the serum placed in screw-cap vials. The vials were stored at -20 C until assayed.

Radioimmunoassay for testosterone followed the methods of Mongkonpunya and Hafs (1973). Serum samples were run in duplicate for each sample. Testosterone was extracted by vortexing 30 sec with 20 ml of diethyl ether:hexane (1:2). The tubes were then placed in a freezer when the aqueous layer was frozen. The ether layer was decanted from the frozen aqueous layer into a second tube and subsequently dried under nitrogen gas. Antibody was diluted 1:3000 in 0.01 M phosphate saline buffer with 0.1 percent Knox gelatin added. Each tube received 0.2 ml of antibody and was allowed to incubate for 30 mins. Then 0.2 ml of ^{3}H-1,2,6,7-Testosterone (New England Nuclear, 91 C/mM) was incubated with the sample-antibody mixture overnight. One ml of dextran 150 (0.025 percent) and carbon, decolorizing, neutral Norit (Fisher Scientific Co., 0.25 percent) bound the antibody and, therefore, separated the antibody bound testosterone from free testosterone. After

incubation for 10 min and centrifugation, 0.5 ml aliquots of supernatant were placed in counting vials, diluted with scintillation fluid and counted in a liquid scintillation spectrometer (Nuclear Chicago Corp., Mark II).

In order to calculate the amount of testosterone in the samples, three groups of samples were run concurrently with the serum samples. One was a group of recovery tubes (i.e., sera samples to which ^{3}H-1,2-testosterone was added). Testosterone was extracted with ether:hexane as described above, but the frozen samples were decanted into counting vials directly. An average value obtained in this manner was used to correct for testosterone lost during extraction. A second group of samples was used to develop a standard curve. This consisted of tubes containing non-labelled testosterone (0.0 to 1.5 mg). All samples were run in duplicate. Finally, a group of standard sera run in duplicate was used to indicate the reliability of the assay performed at different times during the investigation. Data collected from the scintillation counter were run on a computer program developed by Ekre and Foote (1971) which interpolated the sample values using the standard curve and recovery values to give testosterone in pg per ml of serum.

Photoperiod Experiment

A pineal response to photoperiod experiment was conducted which involved 14 adult male ground squirrels collected in the summer of 1974. These animals were placed in an environmental chamber on

July 23, 1974, with an L:D, 14:10 photoperiod from 6:00 a.m. to 8:00 p.m. at room temperature. The squirrels were housed in National traps with food and water supplied ad libitium (supplemented with lettuce greens). The light source was four Champion 72 T12 cool white fluorescent bulbs (Standard USA). After two weeks exposure, seven squirrels were sacrificed at 10:00 p.m. on August 6th and seven were sacrificed at 10:00 a.m. on August 7th. All animals were processed as described above except that pineal gland NAT activity was assayed immediately.

Statistical Analysis

A linear correlation analysis was run comparing each of the following factors to all others using a computer program designed by Michael Windham (personal communication). Those factors compared were: days since emergence (date), body weight, paired adrenal weight, pituitary weight, paired testes weight, testes length, testes width, seminiferous tubule diameter, serum testosterone levels, seminal vesicle weight, testicular MAO, anterior hypothalamic MAO, medial hypothalamic MAO, pituitary MAO and pineal NAT activity. This program also calculated by t-test the significant difference of each calculated correlation value from zero correlation. Students' t-test was also used for comparison of mean values.

RESULTS

General Physiology

Emergence and entrance in A/H

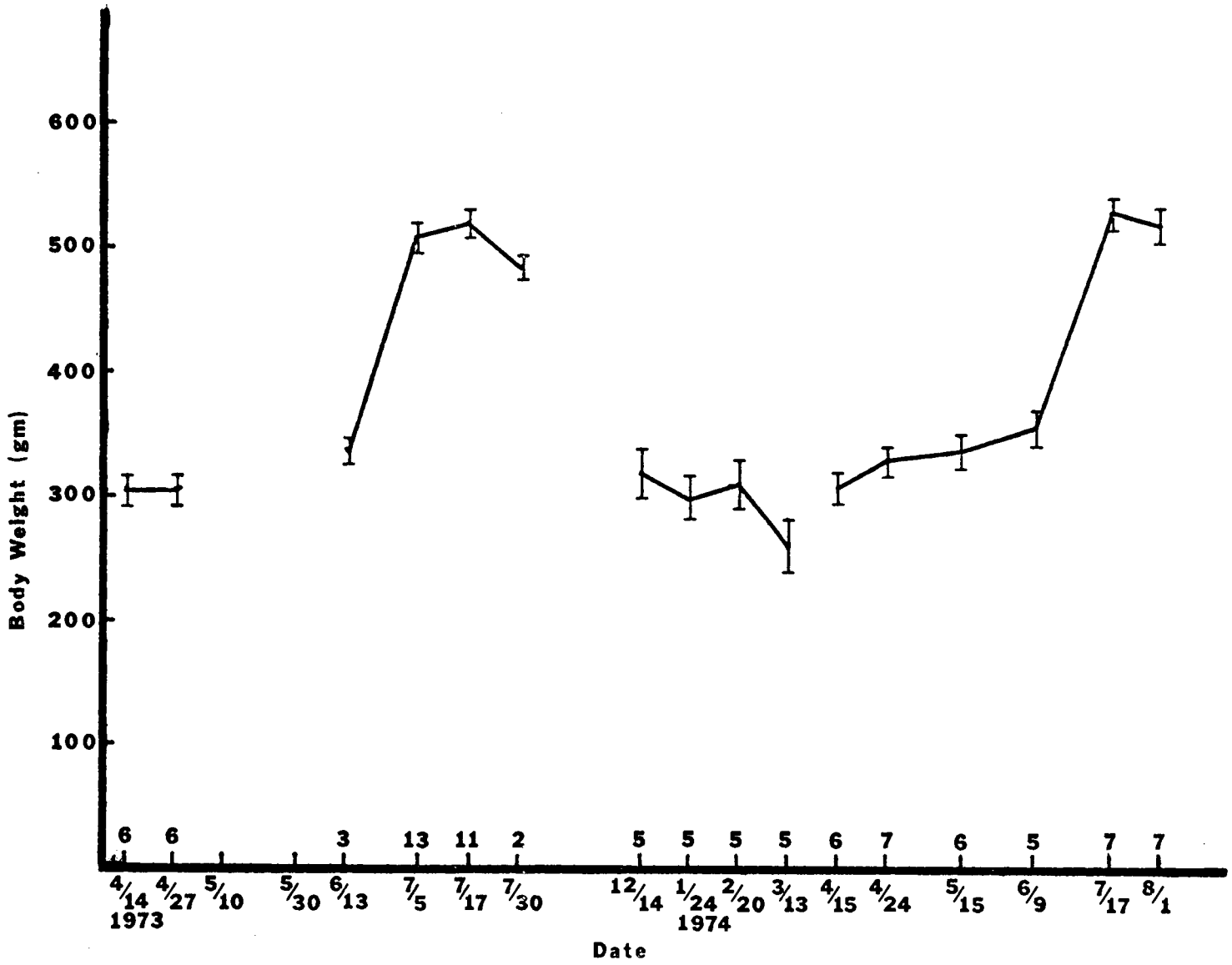
Adult Uinta ground squirrels first emerged at the Utah State University Forestry Camp on April 12, 1973, and on April 8, 1974. The first squirrels of 1973 were quickly followed by a large number of adults. In 1974, however, only one squirrel was seen on the 8th and this day was followed by a few days of cold and cloudy weather. The bulk of the squirrels did not appear until the 12th of April this year.

At the elevation of the Forestry Camp, the last adult male squirrels were captured on July 30th for 1973 and August 1st for 1974. One additional trapping after these dates resulted in capture of only juveniles since all of the adults had apparently entered their hibernacula.

Body weight

The mean body weight for the emerging males collected was 302 $\pm$ 13 g in 1973 and 301 $\pm$ 15 g in 1974. Body weight increased slowly through May and June, then increased sharply in late June and July (Fig. 1). In both years, the average weight just before entering A/H was above 500 g. This weight gain was almost entirely fat deposition since the body mass, depleted of fat by ether extraction, did not increase appreciably over the activity period (James Gessaman, personal communication). The body weights for May 5th and May 30th, 1973, were inadvertantly not taken, resulting in a discontinuous line for 1973.

Figure 1. Body weight changes during the spring-summer seasons and during A/H. The solid line connects the means and the vertical bars are the standard error of the mean. The numbers above the dates on the abcissa are the sample size for each collection date.


Body Weight (gm)
600
500
400
300
200
100
6 6 3 13 11 2 5 5 5 5 6 7 6 5 7 7
4/14 4/27 5/10 5/30 6/13 7/5 7/17 7/30 12/14 1/24 2/20 3/13 4/15 4/24 5/15 6/9 7/17 8/1
1973
1974
Date

Adrenal weight

Although there was some fluctuation and dispersion around each mean, the adrenal weights did not seem to change greatly over the spring-summer (Fig. 2). However, in both years there was a decline during the preA/H period of late July that was statistically significant for 1974 (6/9 to 8/1, $p < 0.05$). Weights collected from squirrels in A/H in the laboratory were uniform during the A/H period and were significantly lower than adrenal weights of preA/H ($p < 0.05$) or emerging ($p < 0.03$) squirrels. A linear regression comparison of adrenal weight and date (days since emergence) for field squirrels gave a correlation coefficient of 0.009 which was non-significant.

Reproductive Physiology

Pituitary weight

Pituitary weights for the spring-summer of 1973 and the A/H period were less than those of the spring-summer of 1974 (Fig. 3). A peak of pituitary weight occurred in May, 1974, that was statistically significant over April 24, 1974 ($p < 0.05$). The values then declined to about the same weight as found in the pituitaries of spring-emerging squirrels.

Testes weight

The pattern of weight changes for the testes was similar during both the 1973 and 1974 spring-summer periods (Fig. 4). At emergence the testes were of maximum size. Testicular regression began immediately as evidenced by the statistically significant decline in late April and

Figure 2. Paired adrenal weight changes during the spring-summer seasons and during A/H. The solid line connects the means for each collection date and the vertical bar represents the standard error of the mean. The numbers above the dates on the abcissa are the sample size for each collection date. Adrenal weights of 7/30/73 were lower than 7/5/73 and weights of 8/1/74 were significantly lower than 6/9/74 ($p < 0.05$). Hibernation weights (12/73-3/74) were significantly lower than 7/30/73 ($p < 0.005$) and 4/15/74 ($p < 0.03$).

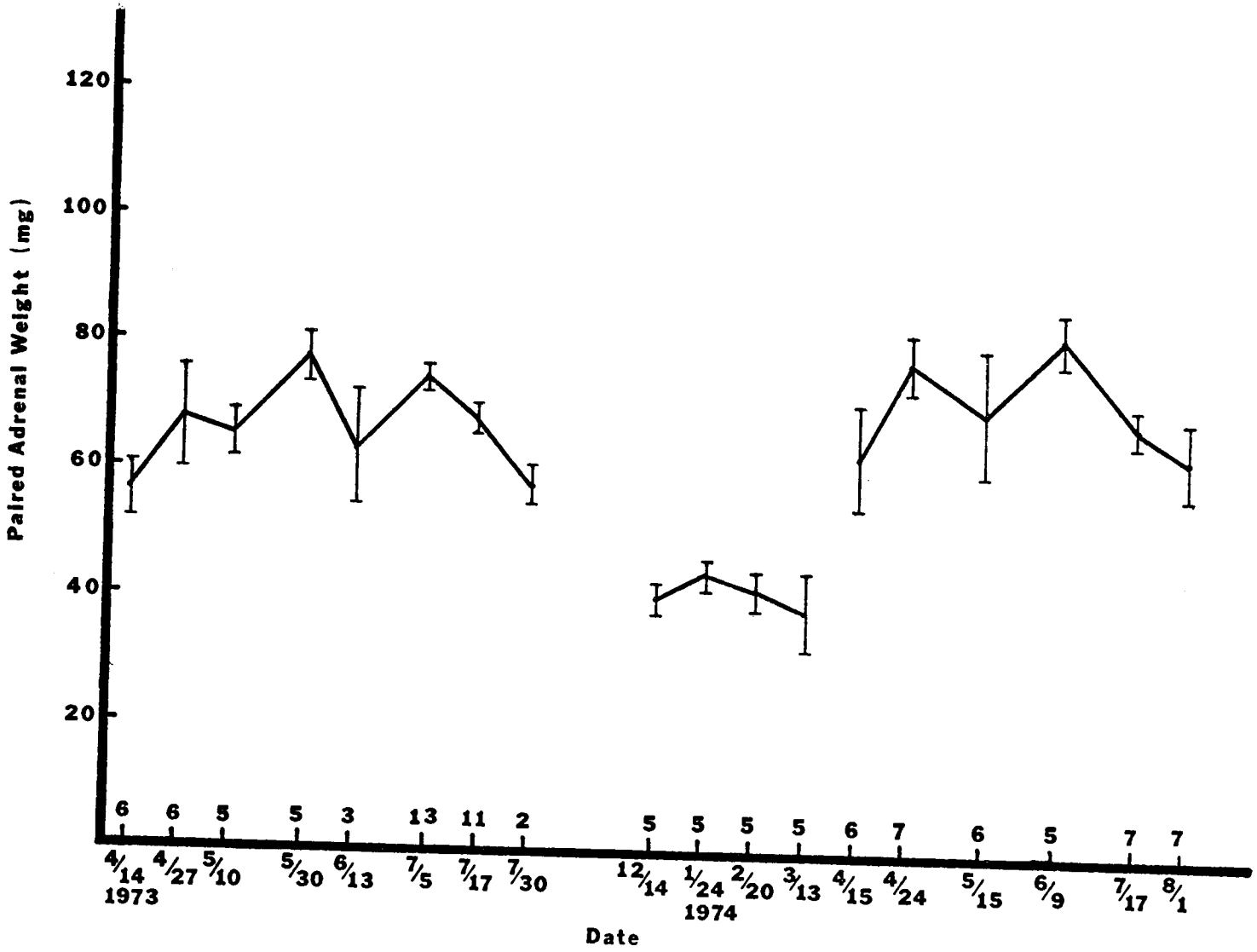

Paired Adrenal Weight (mg)
120
100
80
60
40
20
6 6 5 5 3 13 11 2
5 5 5 5 6 7 6 5 7 7
4/14 1973
4/27
5/10
5/30
6/13
7/5
7/17
7/30
12/14
1/24 1974
2/20
3/13
4/15
4/24
5/15
6/9
7/17
8/1
Date

Figure 3. Pituitary weight changes during the spring-summer seasons and A/H. The solid line connects the means for each collection date and the vertical bars represent the standard error of the mean. The numbers above the dates on the abcissa are the sample size for each collection date. A statistically significant increase in weight occurred on 5/15/74 compared to 4/24/74 ($p < 0.05$).

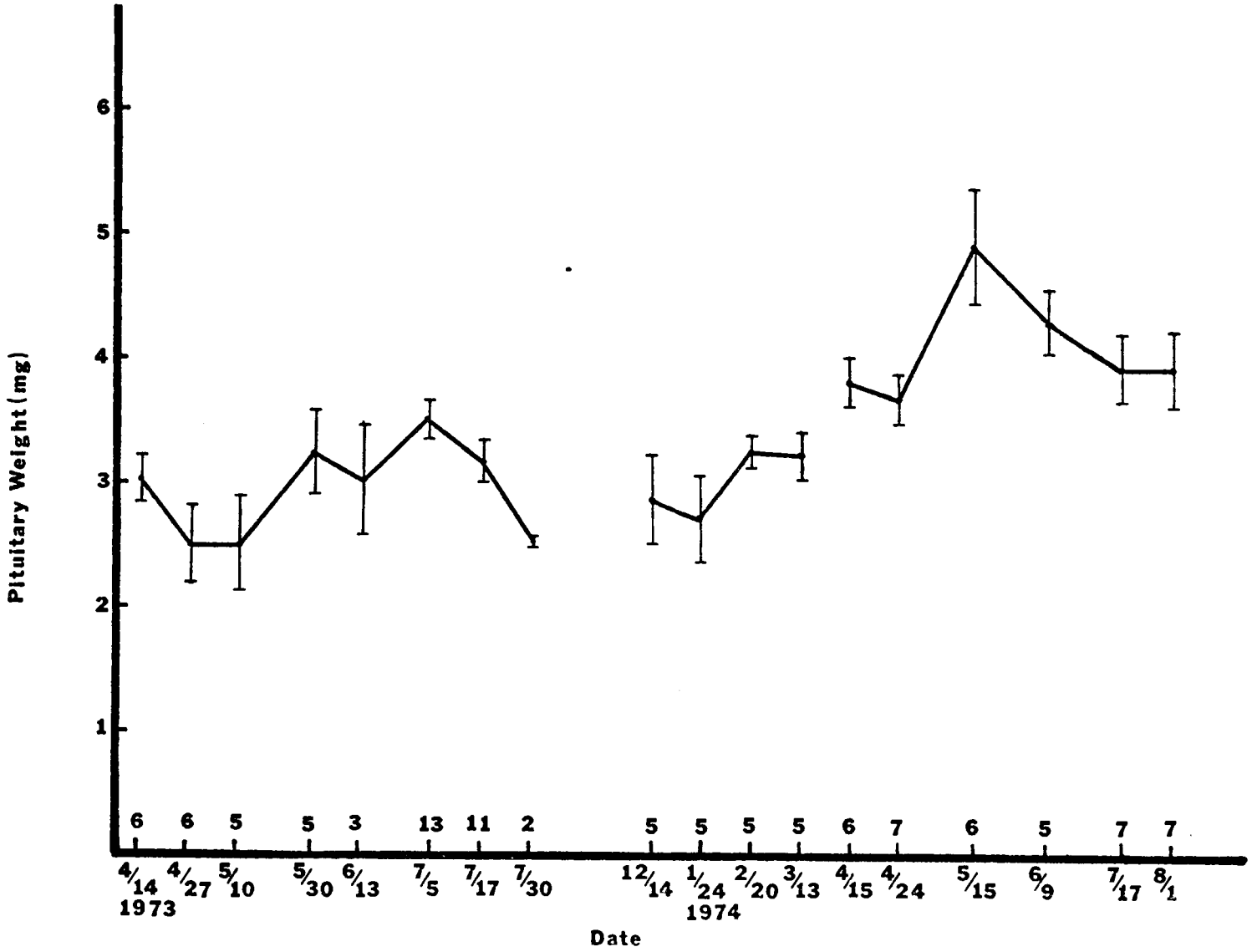
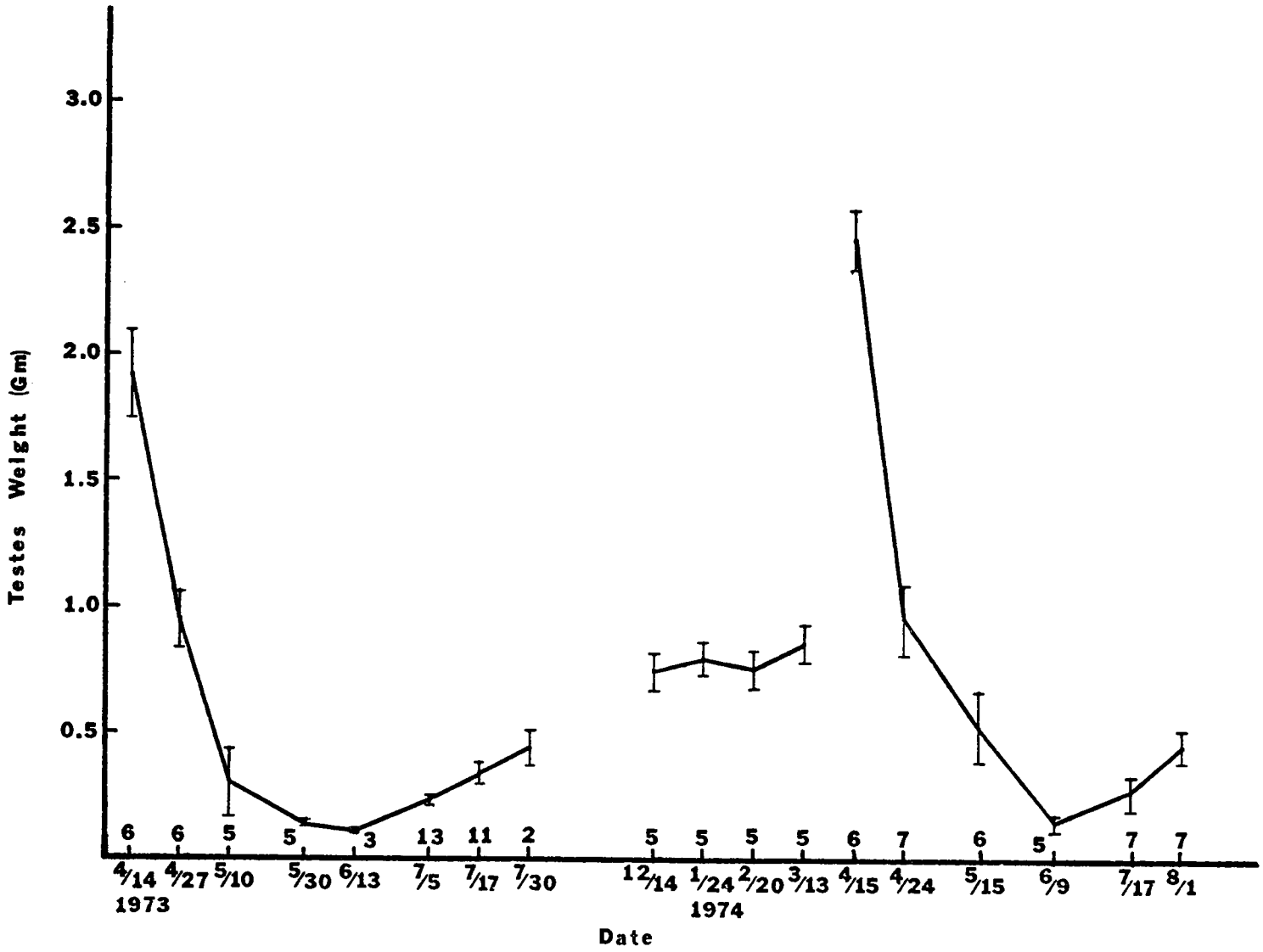

Pituitary Weight (mg)
6
5
4
3
2
1
6 6 5 5 3 13 11 2 5 5 5 5 6 7 6 5 7 7
4/14 4/27 5/10 5/30 6/13 7/5 7/17 7/30 12/14 1/24 2/20 3/13 4/15 4/24 5/15 6/9 7/17 8/1
1973
1974
Date

Figure 4. Paired testes weight changes during the spring-summer seasons and A/H. The solid line connects the means for each collection date and the vertical bars represent the standard error of the mean. The numbers above the dates on the abcissa are the sample size for each collection date. Weights for 6/13/73 were significantly lower than 4/14/73 ($p < 0.001$) and 6/9/74 were significantly lower than 4/15/74 ($p < 0.001$). A statistically significant increase occurred on 7/30/73 over 6/13/73 ($p < 0.02$) and on 8/1/74 over 6/9/74 ($p < 0.003$). Weights for 12/14/73 were increased significantly over 7/30/73 ($p < 0.05$).

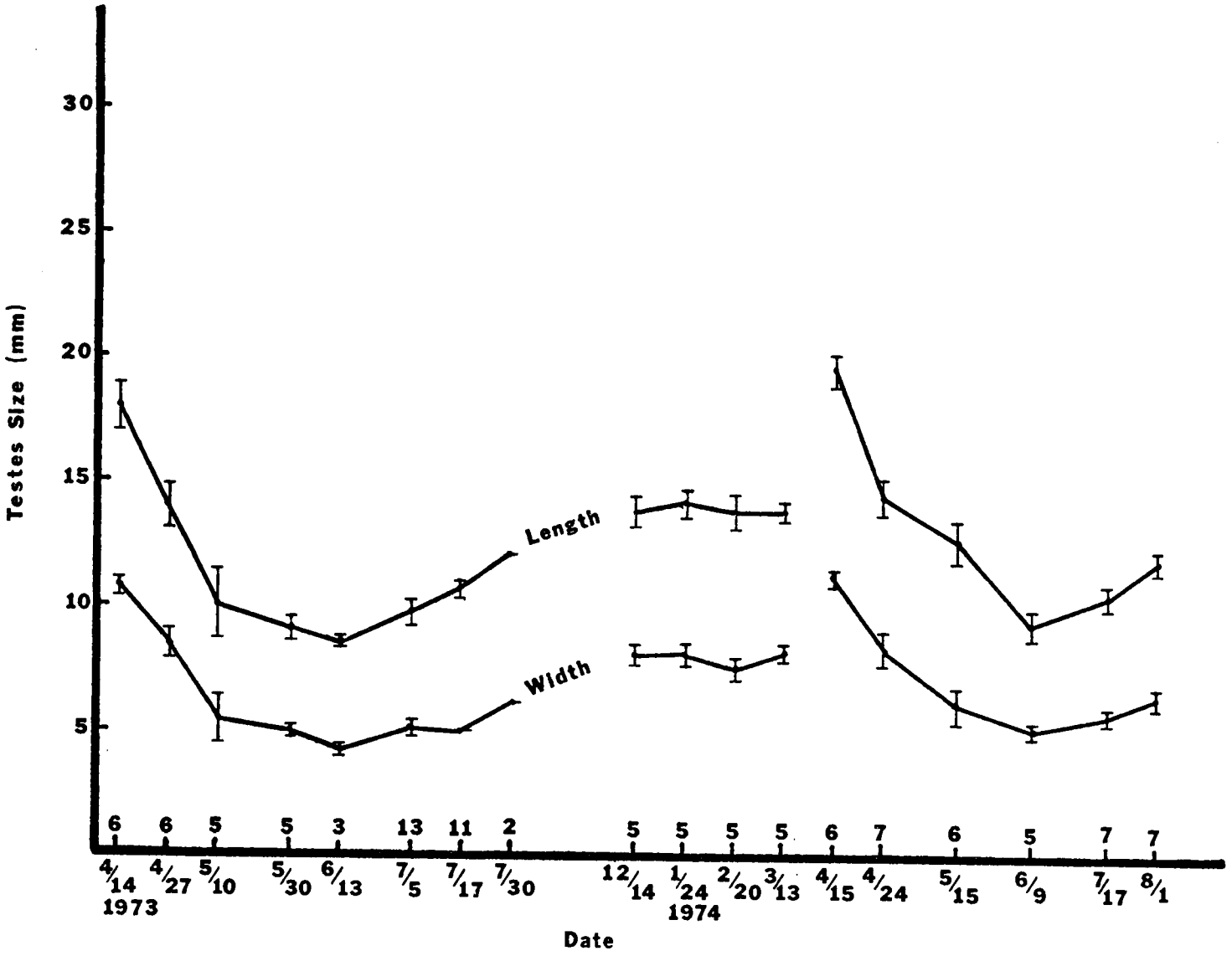

Testes Weight (Gm)
3.0
2.5
2.0
1.5
1.0
0.5
6 6 5 5 3 13 11 2
5 5 5 5 6 7 6 5 7 7
4/14 4/27 5/10 5/30 6/13 7/5 7/17 7/30
1973
12/14 1/24 2/20 3/13 4/15 4/24 5/15 6/9 7/17 8/1
1974
Date

May ($p < 0.001$). In both years, a nadir was reached in mid-June. Thereafter, the size of the testes increased to a statistically significant ($p < 0.02$) level just before entrance into A/H. Testicular weights for the A/H squirrels were significantly higher than the preA/H weights ($p < 0.05$) but had plateaued and were much lower than the emergence weights. Testes weights for the squirrels caught in the field were negatively correlated with the date (days since emergence, $r = -0.69$, $p < 0.001$) and with body weight ($r = -0.52$, $p < 0.001$).

Testes size: Length and width

Testes size (i.e., length and width, Fig. 5) was positively correlated to testicular weights (correlation coefficients were 0.92 for length and 0.93 for width). Testicular size was negatively correlated to date (days since emergence, $r = -0.66$ for length, $r = -0.67$ for width) and to body weight ($r = -0.48$ for length, $r = -0.51$ for width). Testes were maximum in size at emergence. Size decreased rapidly immediately after emergence reaching minimum size in June of both years ($p < 0.001$). The testes increased in size during the preA/H period ($p < 0.005$). During the A/H period, the testes were larger than the preA/H period ($p < 0.08$), but not as large as the emergence levels. These data correlate well with testicular weight (Fig. 4, $r = 0.92$ for length, $r = 0.93$ for width). Therefore, changes in testicular weight during the spring-summer season can be accurately assessed in one individual by measuring the length and width of the testes at different times.

Figure 5. Testicular size changes during the spring-summer seasons and A/H as shown by length and width. The solid lines connect the mean for either length or width for each collection date. The vertical bars represent the standard error of the mean and the numbers above the dates on the abcissa are the sample size for each collection date. Minimum values were reached in June of both years. Values for 5/13/73 were significantly lower than 4/14/73 levels for both length ($p < 0.001$) and width ($p < 0.001$) and 6/9/74 was significantly lower than 4/15/74 for both length ($p < 0.001$) and width ($p < 0.001$). Values for 7/30/73 were significantly greater than 6/13/73 ($p < 0.005$) and 8/1/74 sizes were significantly greater than 6/9/74 ($p < 0.001$). Hibernation values (12/14/73) were greater than preA/H (7/30/73, $p < 0.08$).


Testes Size (mm)
30
25
20
15
10
5
Length
Width
6 6 5 5 3 13 11 2
5 5 5 5 6 7 6 5 7 7
4/14 4/27 5/10 5/30 6/13 7/5 7/17 7/30
1973
12/14 1/24 2/20 3/13 4/15 4/24 5/15 6/9 7/17 8/1
1974
Date

Seminiferous tubule diameter

Histological examination revealed that the diameter of the seminiferous tubules in the testes (Fig. 6) followed the same pattern shown by the testicular weight (Fig. 4) and size (Fig. 5). The diameters were highest in the spring at emergence, declined to a low level in June of each year ($p < 0.005$) then recrudesced during the preA/H period ($p < 0.01$). A/H diameters were higher than the preA/H values ($p < 0.005$) but were lower than the emergence diameters. Seminiferous tubule diameters were positively correlated to testes weight and size ($r = 0.92$ for testes weight, $r = 0.95$ for testes length, and $r = 0.93$ for testes width) and was negatively correlated with date and body weight ($r = -0.64$ for date, and $r = -0.46$ for body weight). These correlations were statistically significant from zero correlation with probabilities less than 0.001.

Testes histology

Histological preparation of the testes revealed that the seminiferous tubules showed marked signs of hypoplasia and regression at the earliest collection date (Fig. 7a). Sperm were present in the lumen of most tubules resulting from maturation depletion of the germinal epithelium. There were some cells of all stages of spermatogenesis present in various tubules (Fig. 7b). However, the spermatogenic layer was low and the cellular associations indistinct. The number of spermatogonia present was greatly reduced. Further hypoplasia and regression, loss of spermatocyte stages, and collapse of the tubule

Figure 6. Seminiferous tubule diameter changes during the spring-summer seasons and A/H. The solid line connects the mean for each collection date while the vertical bars represent the standard error of the mean. The numbers above the dates on the abcissa are the sample size for each collection date. The low values of 6/13/73 were significantly lower than 4/14/73 ($p < 0.005$) and the low values of 6/9/74 were significantly lower than 4/15/74 ($p < 0.001$). The diameters then increased significantly: 7/30/73 was greater than 6/13/73 ($p < 0.001$) and 8/1/74 was greater than 6/9/74 ($p < 0.01$). The diameters during A/H (12/14/73) were significantly greater than the preA/H (7/30/73) period ($p < 0.005$).

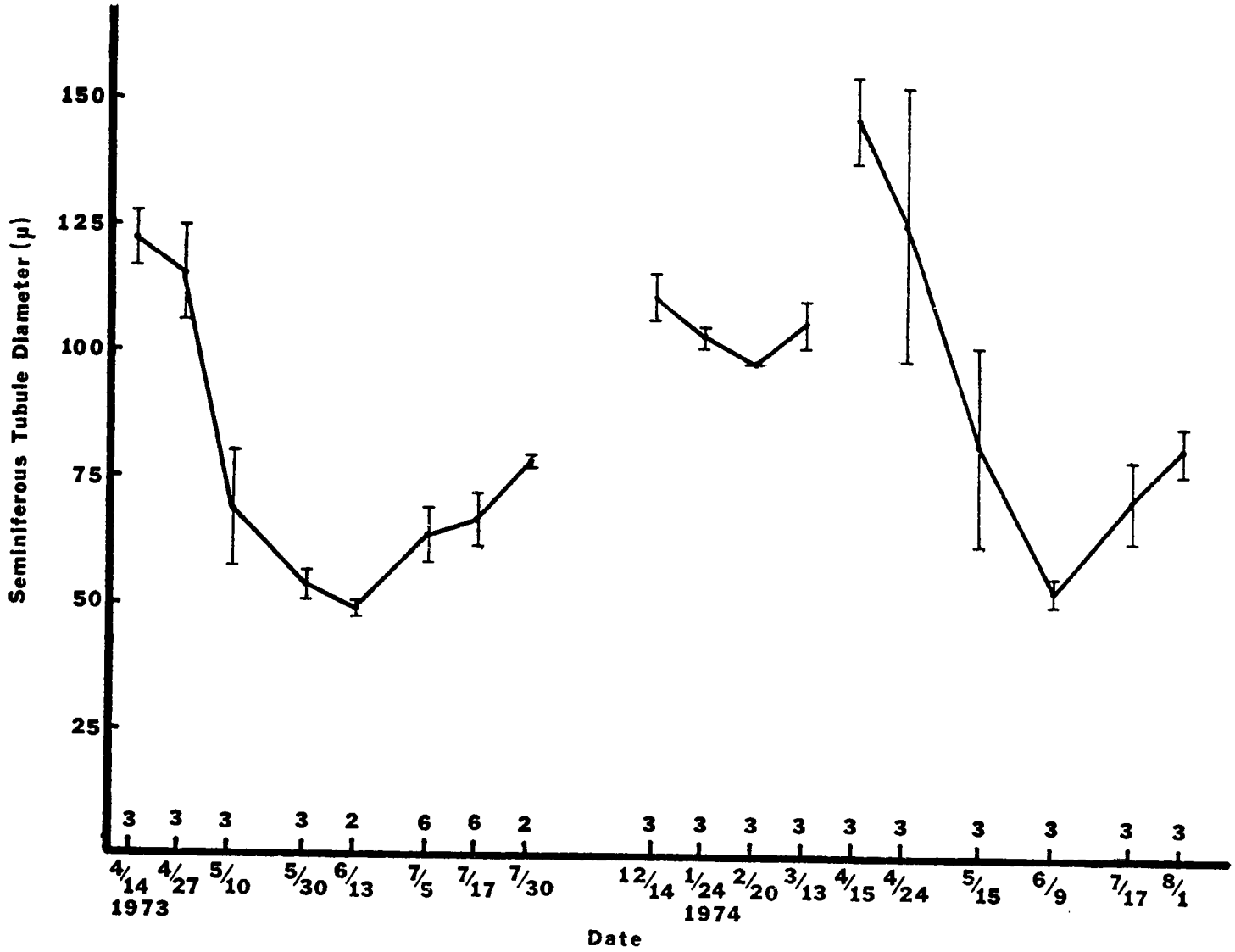
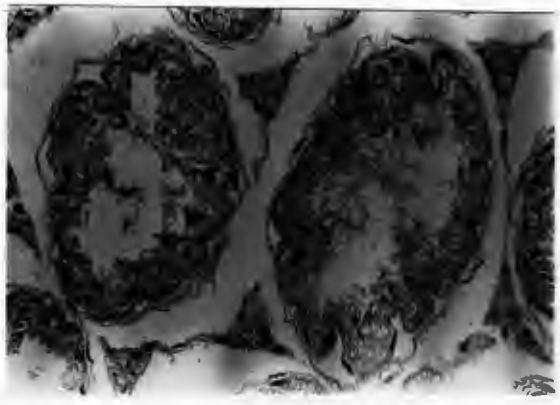
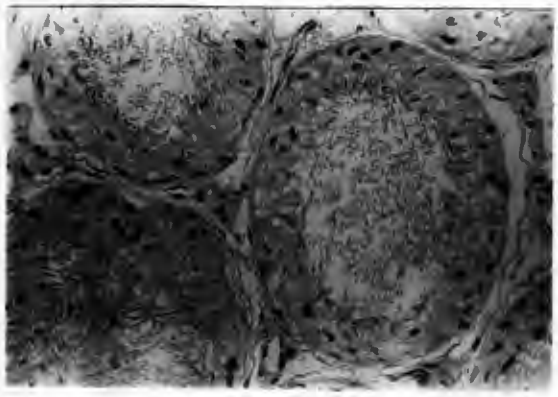

Seminiferous Tubule Diameter (μ)
150
125
100
75
50
25
3 3 3 3 2 6 6 2 3 3 3 3 3 3 3 3 3 3
4/14 4/27 5/10 5/30 6/13 7/5 7/17 7/30 12/14 1/24 2/20 3/13 4/15 4/24 5/15 6/9 7/17 8/1
1973
1974
Date

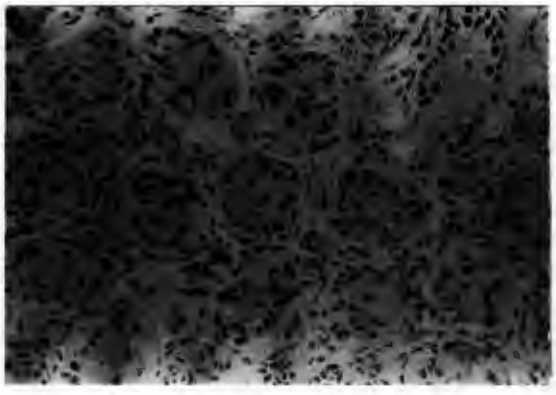
Figure 7. Changes in testicular histology during the spring-summer seasons and A/H. 7a. A section of a testis from a squirrel collected 4/14/73, showing signs of hypoplasia and regression. 7b. Testis of a squirrel collected on 4/15/74 showing presence of sperm and developed germinative layer. 7c. Squirrel testis collected on 6/9/74 showing maximum hypoplasia and regression of tubules and interstitial tissue. 7d. Testis of squirrel collected on 7/29/73 that shows recrudescence of tubule size and germinative layer but little interstitial cell development. 7e. Testis from an A/H squirrel (2/20/74) indicating little development past preA/H level. (All photographs are magnified 235X.)



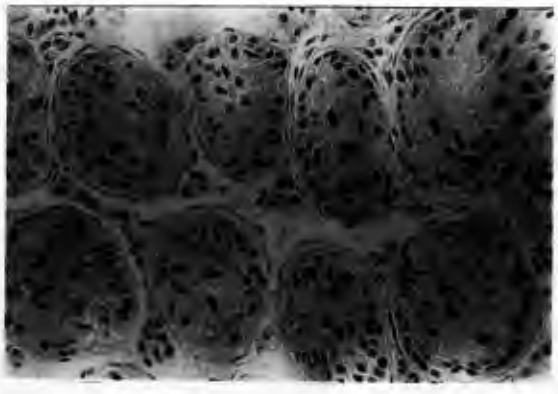
7a



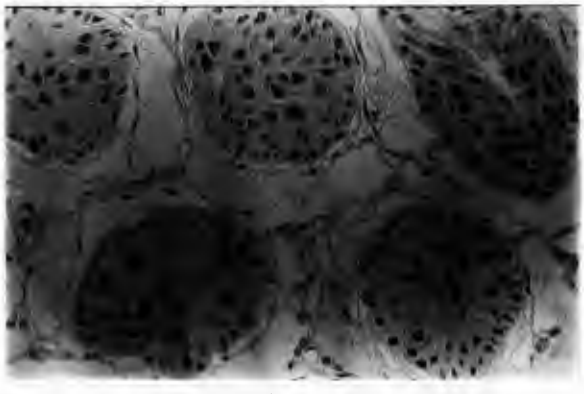
7b



7c



7d



7e

occurred during May and June. Hypoplasia and regression also appeared to occur in the interstitial tissues during May and June.

Seminiferous tubules reached their smallest diameter in June (Fig. 7c) and this corresponded to the maximum degeneration of the spermatogenic elements. The lumens were closed with essentially a single layer of cells around the periphery of the tubules composed of Sertoli cells and spermatogonia. Interstitial tissue was quite sparse. As the testes recrudesced during the preA/H period, a growth of the spermatogenic layers occurred (Fig. 7d). Diameter of the tubules and the number of spermatogonia in the tubules increased so that by the end of this period the histology of the tubules was quite similar to that in a testis of an immature rat. This condition was maintained without appreciable change throughout the A/H period (Fig. 7e). Hyperplasia of the interstitial tissue was not evident during recrudescence of the testes in late summer or during the A/H period. This pattern was the same for both the 1973 and 1974 activity seasons.

Epididymal sperm smears

Spermatozoa were found in the epididymal smears from emergence in April until late May of both 1973 and 1974. The sperm were most plentiful immediately after emergence and declined in abundance thereafter. Three out of five squirrels gave a positive epididymal sperm smear on May 30, 1973. The testes contained sperm in the seminiferous tubules from emergence through mid-May. No spermatozoa were seen in the seminiferous tubules from late May through the A/H period nor in the epididymides from June through the A/H period.

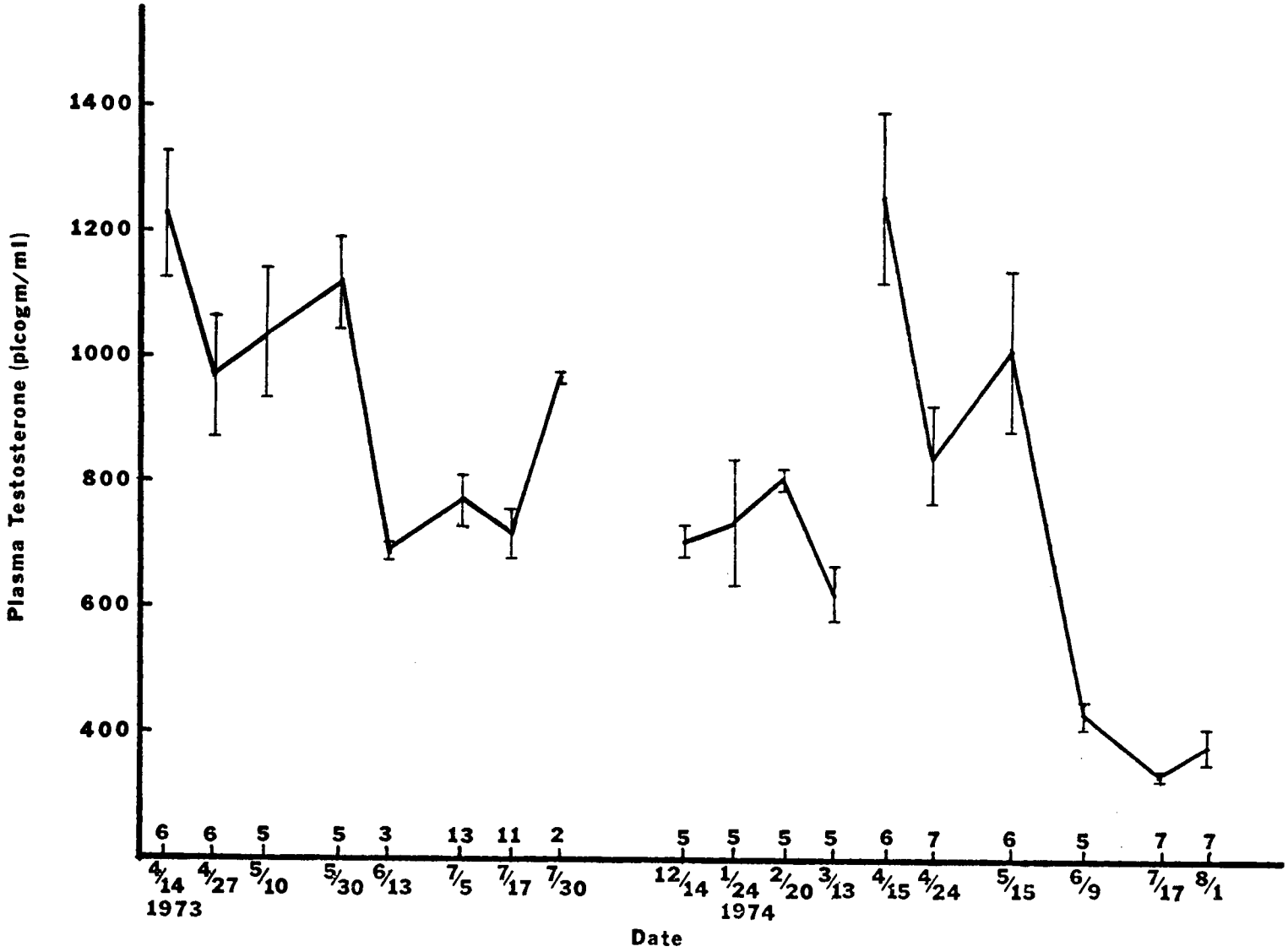
Plasma testosterone

In both years, the levels of testosterone in the blood at emergence were the highest recorded (Fig. 8). The levels then dropped to a lower level in late April. This decline approached statistical significance in 1973 ($p < 0.08$) and was highly significant in 1974 ($p < 0.005$). The increase during May of both years was not statistically significant. The May increase was followed in both years by a statistically significant decrease to a low level in June ($p < 0.005$) that was maintained through July. The low level of the preA/H period in 1974, however, was much lower than in 1973. Although the squirrel testosterone assays were run in three separate groups, values of serum from a non-pregnant cow, an ovariectomized cow, a pregnant cow and a steer from each group were essentially the same for the three assays. Therefore, the difference in blood levels in June and July of 1973 and 1974 cannot be explained by differences inherent in the assay.

In late July of 1973, a significant increase ($p < 0.02$) in testosterone levels occurred, but this did not occur in 1974. Although statistically significant, the 1973 increase was based on two individuals and may not reflect a true pattern. The A/H values did not support a preA/H increase in secretion of testosterone since they were low and essentially plateaued. The slight decrease from the December value to the March value was not statistically significant.

Plasma testosterone levels for the squirrels caught in the field were negatively correlated with date ($r = -0.62$, $p < 0.001$) and with body weight ($r = -0.54$, $p < 0.001$). Plasma levels were positively

Figure 8. Plasma testosterone levels during the spring-summer seasons and A/H. The solid line connects the mean for each collection date and the vertical bars represent the standard error of the mean. The numbers above the dates on the abcissa are the sample size for each collection date. The decline from 4/14/73 to 4/27/73 approached statistical significance ($p < 0.08$) while the decline from 4/15/74 to 4/24/74 was significant ($p < 0.005$). The decline from 5/30/73 to 6/13/73 and from 5/15/74 to 6/9/74 was significant ($p < 0.005$). A significant increase occurred from 7/17/73 to 7/30/73 ($p < 0.02$) but a similar increase did not occur in 1974.


Plasma Testosterone (picogm/ml)
1400
1200
1000
800
600
400
6 6 5 5 3 13 11 2
5 5 5 5 6 7 6 5 7 7
4/14 4/27 5/10 5/30 6/13 7/5 7/17 7/30
1973
12/14 1/24 2/20 3/13 4/15 4/24 5/15 6/9 7/17 8/1
1974
Date

correlated with testicular weight ($r = 0.61$, $p < 0.001$), and testicular size ($r = 0.54$, $p < 0.001$, for length; $r = 0.55$, $p < 0.001$ for width).

Seminal vesicle weights

The seminal vesicles had their greatest weight immediately after the spring emergence in April of both years (Fig. 9). The weights then dropped off rapidly during May of both years and by June had reached a minimal value ($p < 0.001$). The seminal vesicles did not recrudesce as did the testes in the preA/H and A/H periods. The weights of the seminal vesicles remained at a low level throughout these periods in both 1973 and 1974. Linear regression comparison of seminal vesicle weights with date and body weight resulted in significant negative correlations ($r = -0.81$, $p < 0.001$ for date; $r = -0.72$, $p < 0.001$ for body weight). Statistically significant positive correlations resulted from comparisons with testicular weight ($r = 0.70$, $p < 0.001$), testes size ($r = 0.78$ for length, $r = 0.80$ for width, both $p < 0.001$), and testosterone ($r = 0.53$, $p < 0.001$).

MAO

Testicular MAO

Testicular MAO (presented as the counts per minute per mg of tissue against the date) activity was quite high when the testes were developed at emergence (Fig. 10). The values decline during the mid-summer and preA/H period and did not rise with the recrudescence of the testes during the preA/H or A/H period. The slight increase in July of 1973 was not statistically significant ($p < 0.50$) nor was there any significant change during A/H.

Figure 9. Seminal vesicle weights during the spring-summer seasons and A/H. The solid line connects the mean for each collection date and the vertical bars represent the standard error of the mean. The numbers above the dates on the abcissa are the sample size for each collection date. The decline in both years from emergence collection (4/14/73 and 4/15/74) to June (6/13/73 and 6/9/74) were statistically significant ($p < 0.001$). No significant change occurred during the preA/H periods or the A/H period December through March.

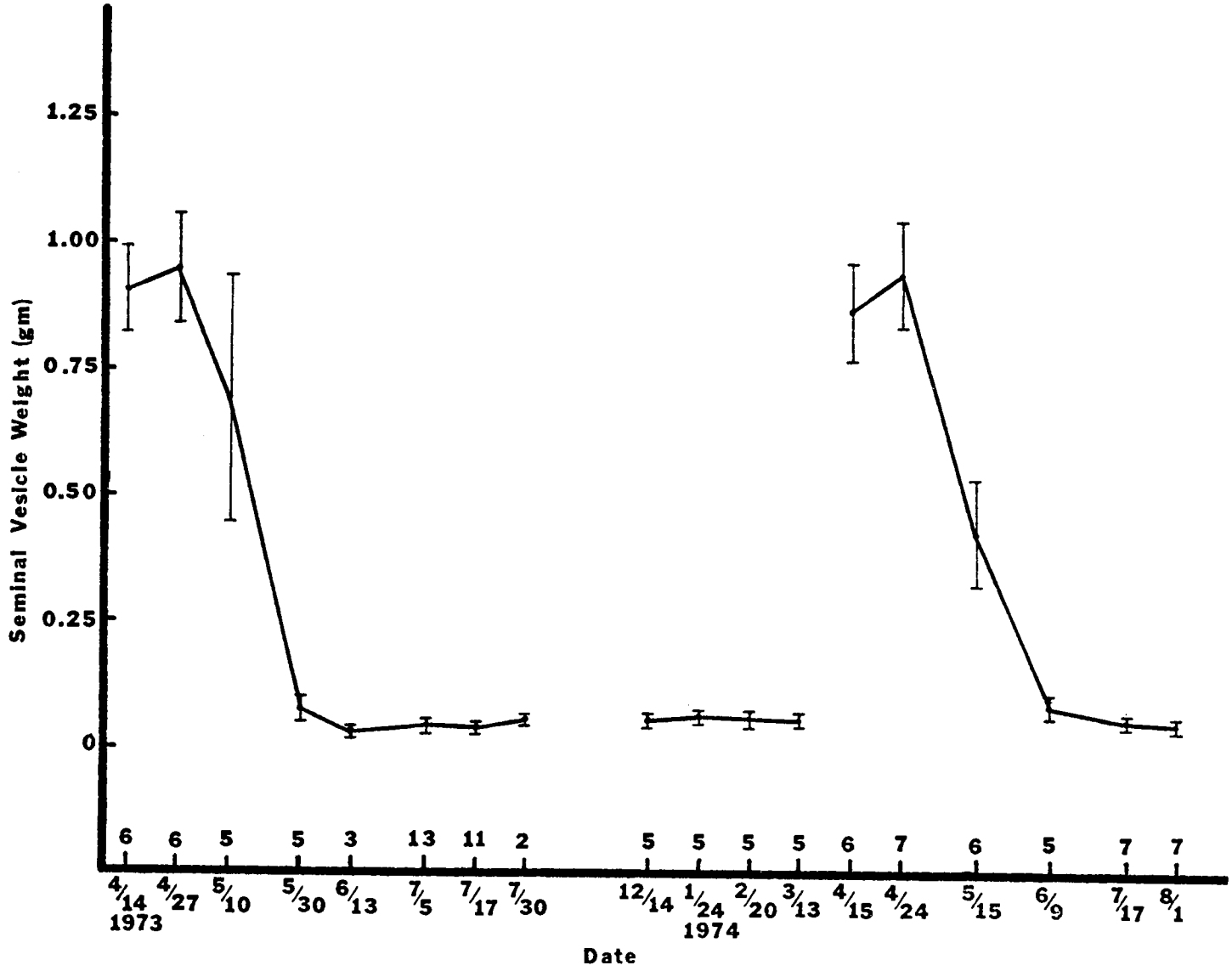
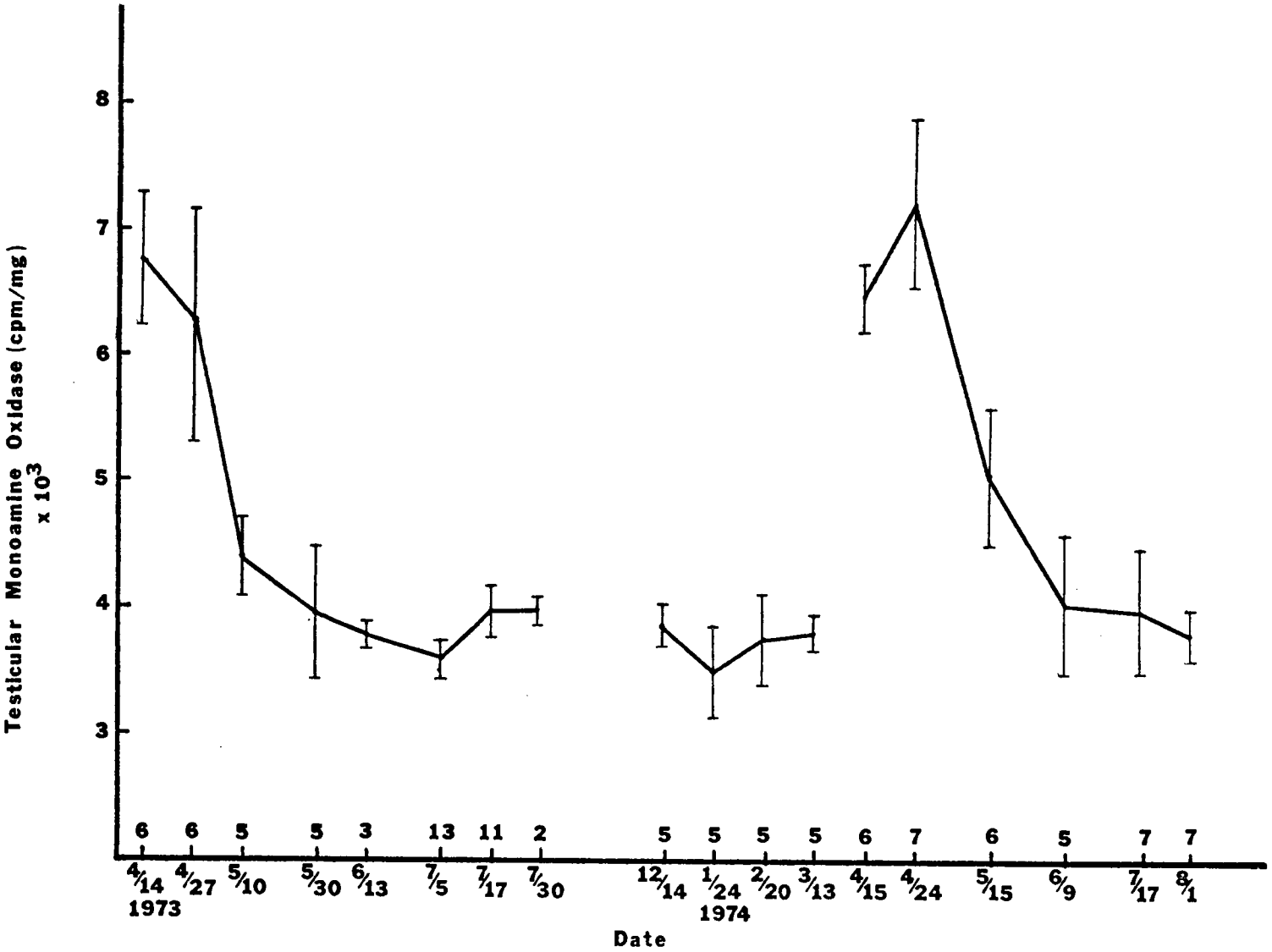

Seminal Vesicle Weight (gm)
1.25
1.00
0.75
0.50
0.25
0
6 6 5 5 3 13 11 2 5 5 5 5 6 7 6 5 7 7
4/14 1973
4/27
5/10
5/30
6/13
7/5
7/17
7/30
12/14
1/24 1974
2/20
3/13
4/15
4/24
5/15
6/9
7/17
8/1
Date

Figure 10. Testicular monoamine oxidase activity changes during the spring-summer seasons and A/H expressed as counts per minute per milligram of tissue. The solid line connects the mean for each collection date and the vertical bars represent the standard error of the mean. The numbers above the dates on the abcissa are the sample size for each collection date. The declines from 4/14/73 to 7/5/73 and from 4/15/74 to 7/17/74 were both statistically significant ($p < 0.001$). The slight increase of 7/17/73 and 7/30/73 over 7/5/73 was not statistically significant ($p < 0.50$).

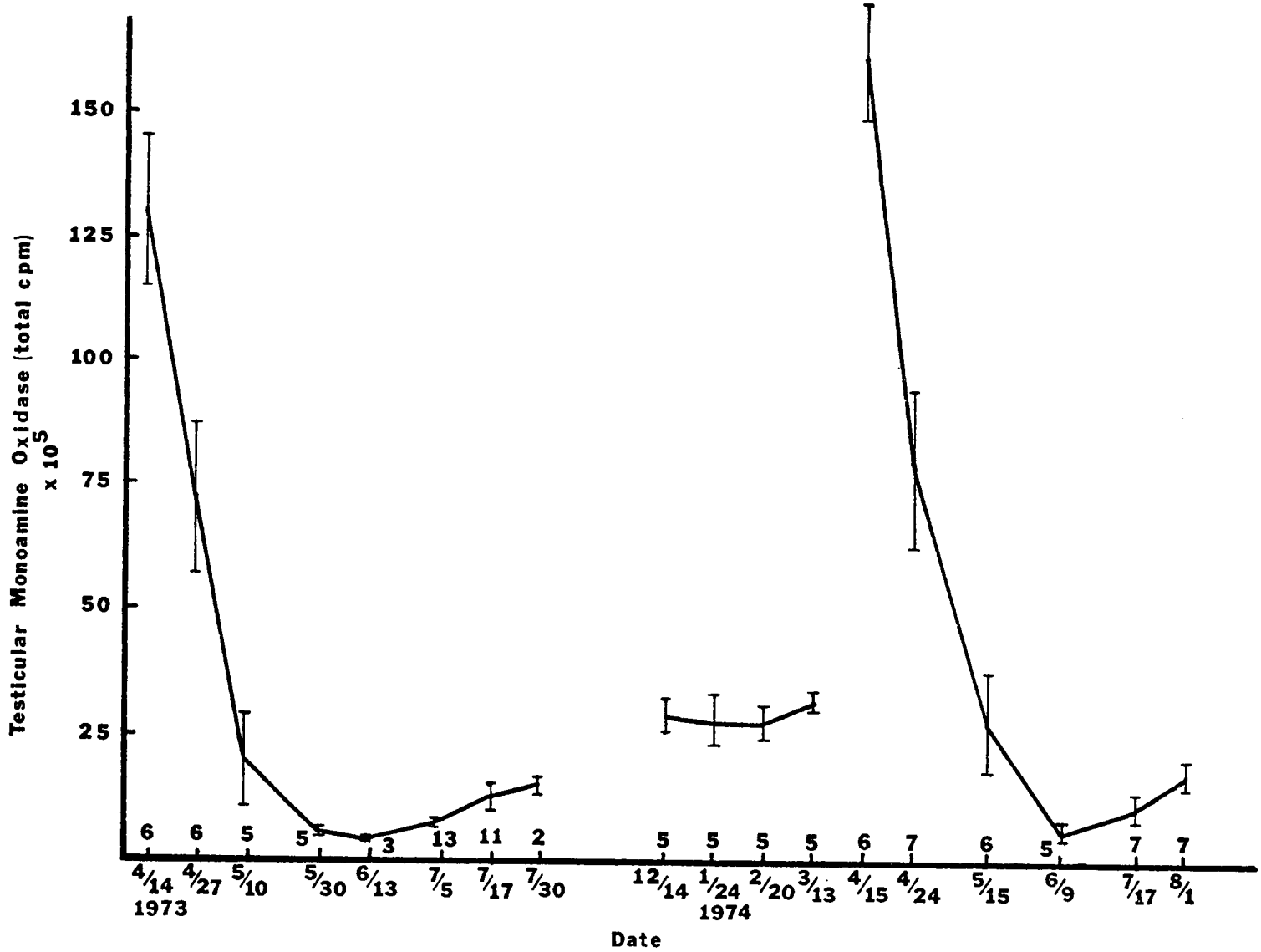

Testicular Monoamine Oxidase (cpm/mg) x 10^3
8
7
6
5
4
3
6 6 5 5 3 13 11 2 5 5 5 5 6 7 6 5 7 7
4/14 4/27 5/10 5/30 6/13 7/5 7/17 7/30 12/14 1/24 2/20 3/13 4/15 4/24 5/15 6/9 7/17 8/1
1973
1974
Date

When the testicular MAO was expressed as the total counts per minute per pair of testes, however, the pattern was different (Fig. 11). The values were high at emergence and began to decline immediately. The activity was at its lowest point in June of both years ($p < 0.001$), but during the preA/H period, a statistically significant increase in activity occurred during both years compared to the low value in June ($p < 0.01$). The activity during the A/H period plateaued, but was at a statistically significant higher level than the preA/H values ($p < 0.001$). This pattern of total MAO activity change corresponded closely to the testicular size changes since the correlation coefficient for total MAO compared to testicular weight was 0.96, highly significant ($p < 0.001$).

Pituitary MAO activity

Pituitary MAO was assayed during the A/H period and for the 1974 spring-summer season (Fig. 12). The increase that occurred during the A/H period approached statistical significance ($p < 0.06$). The mean activity level on 12/14/74 was significantly lower than the 4/15/74 emergence level ($p < 0.03$). A significant increase over the emergence level (4/15/74) occurred on 4/24/74 ($p < 0.05$) and then a significant drop in activity level from 4/24/74 occurred on 5/15/74 ($p < 0.01$). The activity level then increased during the preA/H period. The mean activities on 7/17/74 and 8/1/74 were significantly higher than the 5/15/74 value ($p < 0.05$ and $p < 0.01$, respectively). A linear correlation analysis comparing body weight and pituitary MAO activity resulted

Figure 11. Testicular MAO activity changes during the spring-summer seasons and A/H period expressed as counts/minute/paired testes. The solid line connects the mean for each collection date and the vertical bar represents the standard error of the mean. Declines in activity from 4/14/73 to 6/13/73 and from 4/15/74 to 6/9/74 were highly significant ($p < 0.001$). Increases occurred during the preA/H period which were statistically significant (7/30/73 to 6/13/73, $p < 0.01$; 8/1/74 to 6/9/74, $p < 0.01$). Hibernation activity levels (12/14/73 through 3/13/74) were significantly higher than the preA/H level of 7/30/73 ($p < 0.001$).


Testicular Monoamine Oxidase (total cpm)
x 10^5
150
125
100
75
50
25
6 6 5 5 3 13 11 2
5 5 5 5 6 7 6 5 7 7
4/14 4/27 5/10 5/30 6/13 7/5 7/17 7/30
1973
12/14 1/24 2/20 3/13 4/15 4/24 5/15 6/9 7/17 8/1
1974
Date

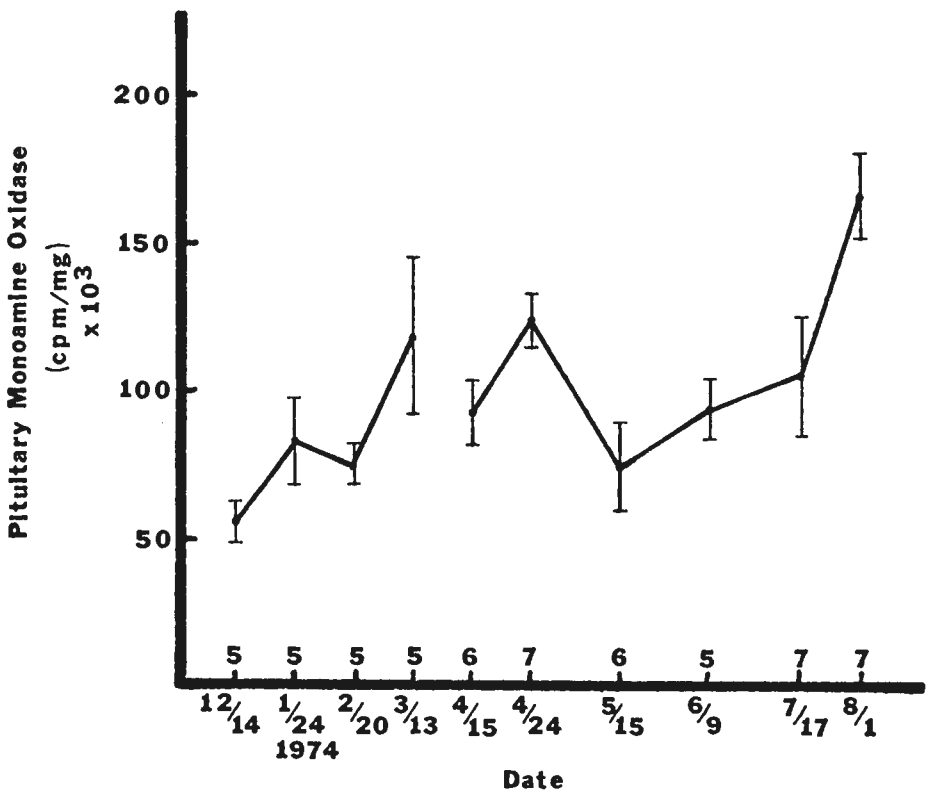


Figure 12. Pituitary MAO activity during A/H and 1974 spring-summer season. The solid line connects the mean for each collection date and the vertical bars represent the standard error of the mean. Numbers above the dates on the abcissa are the sample size for each collection date. The 12/14/73 A/H activity was significantly lower than the emergence activity on 4/15/74 ($p < 0.05$). The increase from 4/15 to 4/24 was significant ($p < 0.05$) and the decrease from 4/24 to 5/15 was also significant ($p < 0.01$). A significant increase occurred from 5/15 to 8/1 ($p < 0.01$).

in a significant correlation coefficient of ($r = +0.51$, $p < 0.005$). No correlation was found between pituitary weight and pituitary MAO.

Hypothalamic MAO activity

Except for one collection date, 2/20/74, the anterior hypothalamic MAO activity was consistently lower than the medial hypothalamic activity (Fig. 13). There was, however, no statistically significant difference between the anterior and medial hypothalamic activity at any collection date. A significant increase occurred in the activity of both anterior and medial hypothalamic areas between 12/14/73 and 1/24/74 ($p < 0.05$). A decrease occurred between 1/24/74 and 2/20/74 that was statistically significant for the medial hypothalamic activity ($p < 0.05$). The activity level for both hypothalamic areas in the emerging squirrels was high. However, this was not a significant increase over the last A/H period sample in March ($p < 0.10$). Immediately following emergence, the activity levels of both hypothalamic areas dropped to a significantly lower level ($p < 0.05$) compared to emergence values. Both values remained low during May and June, increased significantly in July ($p < 0.01$) over the June levels, then remained at the higher level through 8/1/74. The correlation coefficient between anterior and medial hypothalamic MAO was $r = +0.86$, highly significant ($p < 0.001$).

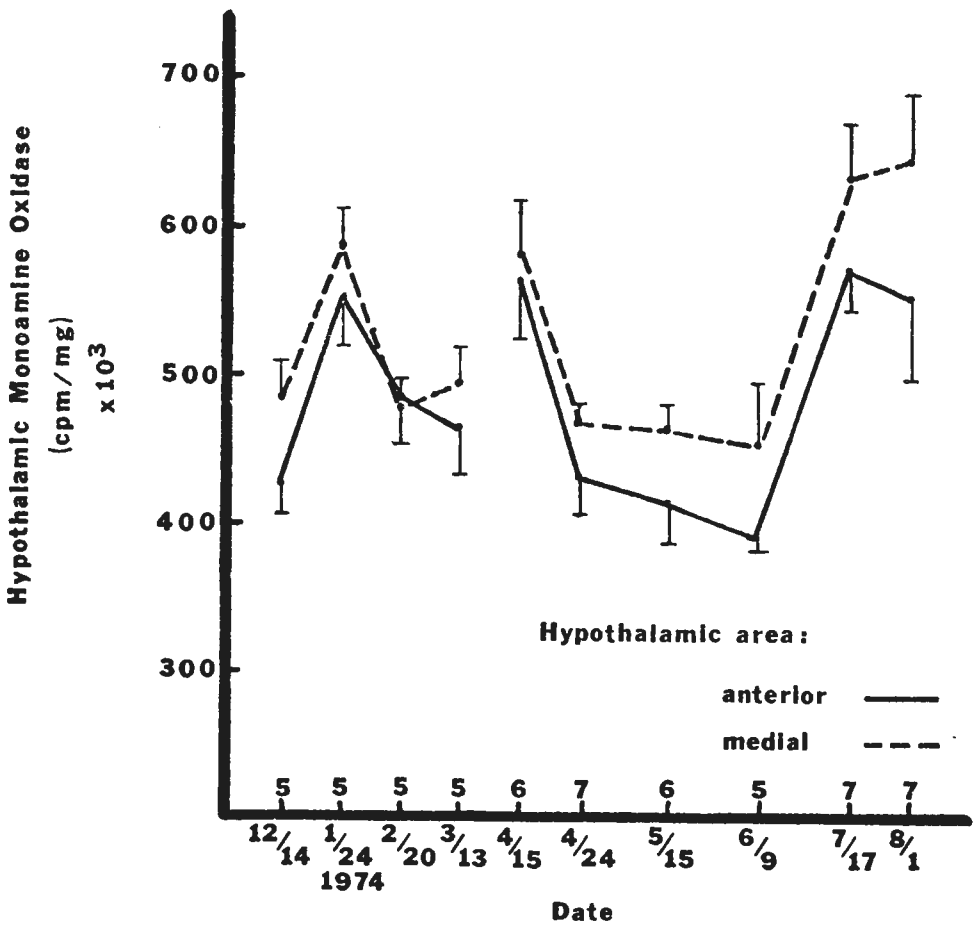


Figure 13. Hypothalamic MAO activity during A/H and the 1974 spring-summer season. The solid line connects the mean value for each collection date for the anterior hypothalamus and the dashed line connects the mean value for each collection date for the medial hypothalamus. The vertical bars represent the standard error of the mean. Numbers above the dates on the abcissa are the sample size for each collection date. The increase in activity during A/H from 12/14/73 to 1/24/74 was significant ($p < 0.05$) for both hypothalamic areas. The decline from 1/24 to 2/20 was significant for the medial hypothalamic area ($p < 0.05$). The activity level from 4/15 to 4/24 was a significant decline in activity for both areas ($p < 0.05$). A significant increase for both hypothalamic areas occurred from 6/9 to 7/17 ($p < 0.01$).

Pineal NAT

Photoperiod experiment

No statistically significant difference occurred between the night and day activity levels (Table 1) with either expression of pineal activity although the activity per mg of tissue approached significance ($p < 0.07$). However, in both expressions, the activity of the pineal NAT of the night group was greater than the activity of the day group.

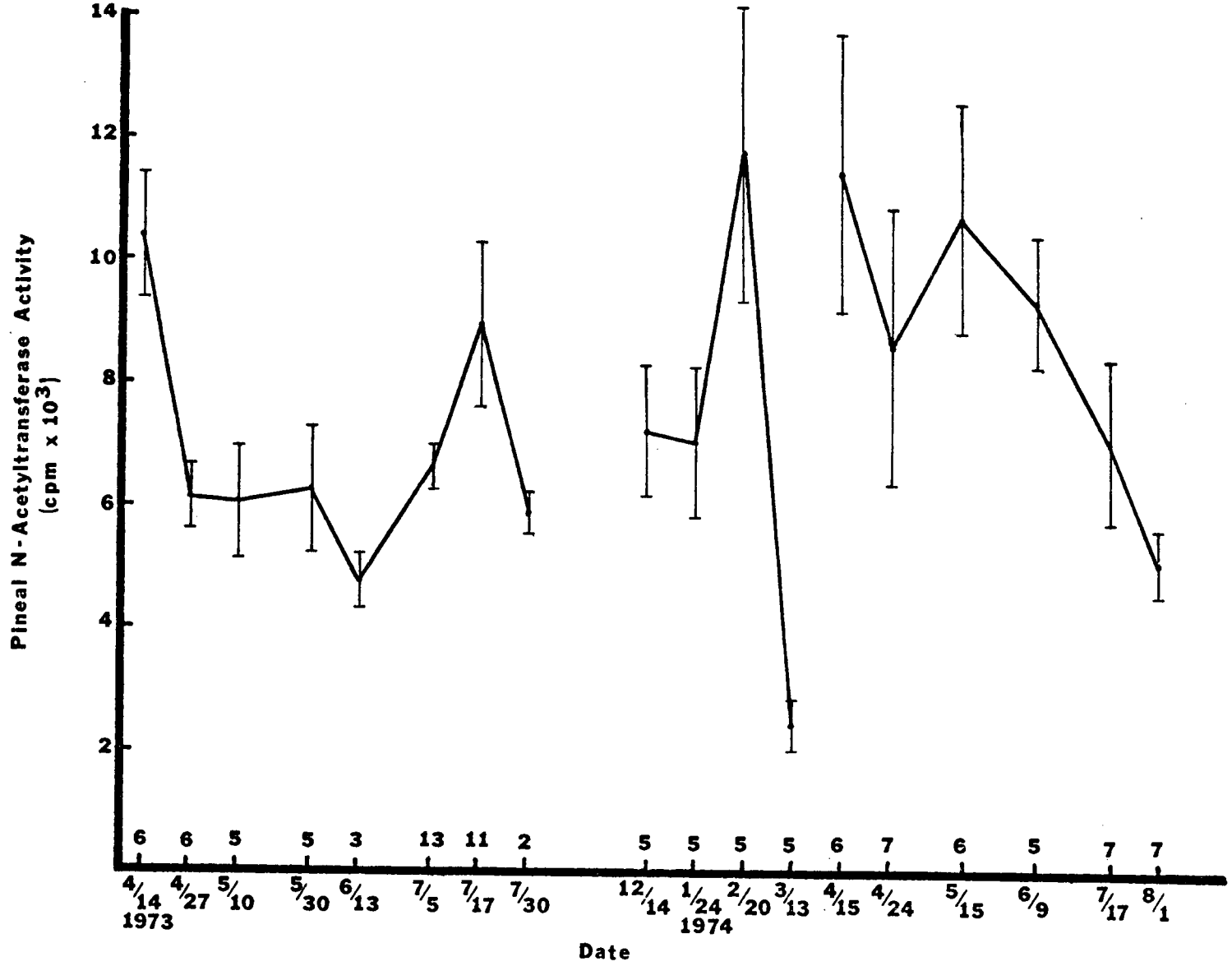
Table 1. Diurnal changes in pineal NAT activity (counts per 10 minutes per pineal or counts per 10 minutes per mg tissue, ± standard error of the mean)

Time	C/10min/pineal	C/10min/mg
10:00 p.m. August 6	13,535 ± 3854	7,894 + 1435
10:00 a.m. August 7	6,872 ± 724	4,728 ± 557
	$p < 0.12$	$p < 0.07$

Seasonal change in pineal NAT activity

The patterns for pineal NAT activity for 1973 and 1974 are different (Fig. 14). During the 1973 spring-summer season, the pineal activity was high at emergence and declined immediately. The drop in activity from 4/14/73 to 4/27/73 was statistically significant ($p < 0.02$). The activity then plateaued through June with no change in activity.

Figure 14. Pineal NAT activity changes during the spring-summer seasons and A/H period. The solid line connects the mean for each collection date and the vertical bar represents the standard error of the mean. Numbers above the dates on the abcissa are the sample size for each collection date. The 1973 decline from 4/14 to 4/27 was statistically significant ($p < 0.02$). The A/H decline from 2/20/74 to 3/13/74 was significant ($p < 0.01$). The decline from 4/15/74 to 8/1/74 was significant ($p < 0.02$).


Pineal N-Acetyltransferase Activity
(cpm x 10³)
14
12
10
8
6
4
2
6 6 5 5 3 13 11 2
5 5 5 5 6 7 6 5 7 7
4/14 1973
4/27
5/10
5/30
6/13
7/5
7/17
7/30
12/14
1/24 1974
2/20
3/13
4/15
4/24
5/15
6/9
7/17
8/1
Date

During July, NAT activity rose through the 5th and 17th, then declined again on the 30th. Neither the increase from 6/13/73 to 7/5/73, from 6/13/73 to 7/17/73, nor from 7/5/73 to 7/17/73, nor the decrease from 7/17/73 to 7/30/73 were significant, however.

The A/H NAT activity levels in December and January were not statistically different from the prehibernation July values. A non-significant increase in February occurred. A very sharp, statistically significant, drop in activity occurred in March just prior to emergence ($p < 0.01$).

In April of 1974, pineal NAT activity levels were again high at emergence and this was followed by a decline. In contrast to 1973, however, this decline was not statistically significant. The values tended to decline throughout the year so that by 8/1/74, the NAT activity was significantly below the 4/15/74 emergence level ($p < 0.02$). No increase was seen before entrance into A/H.

DISCUSSION

General Physiology

Adult male squirrels emerged in 1973 and 1974 in the same time period as those emerging in 1965, 1966 and 1967 (Walker, 1968). Walker (1968) reported dates of April 16, March 28, and March 21 for the years 1965, 1966 and 1967, respectively. Walker (1968) also reported dates of immergence of adult male and yearling male squirrels into A/H. Years with late average emergence (1965) had later average immergence (July 22 for adult males, July 28 for yearling males) while years with early emergence (1966) had earlier immergence dates (July 3 for adult males, July 15 for yearling males). Data presented here agree with this finding in that the last adult males (including yearling males) were collected during the last of July, 1973, or the first of August, 1974.

Emergence body weights of 302 g for 1973 and 301 g for 1974 also agree closely with data presented by Walker for squirrels emerging in April (1968). Average body weight for emerging adult male squirrels for 1965 was 302 g while weights in 1966 and 1967 were considerably higher (348 and 351 g respectively)(Walker, 1968). These data tend to corroborate the hypothesis of D. F. Balph (personal communication) that Uinta ground squirrels arouse from A/H at about the same time each year (around March 15), but do not emerge until conditions are favorable. During this interim between arousal and emergence, the squirrel rapidly uses stored energy because of an increased level of metabolic

activity. With a longer interim period, body weight at emergence is lower. In 1965, 1973, and 1974, with emergence beginning in mid-April, the body weights were considerably lower than in years when squirrels began to emerge in March (1966 and 1967).

Body weight did not increase greatly during the breeding season, but increased rapidly in late June and early July. This agreed well with published data on other ground squirrels (McKeever, 1964; Mrosovsky and Fisher, 1970; Iverson and Turner, 1972) and with data on the Uinta ground squirrels (Knopf, 1973). As in other studies, maximum weights were attained just prior to immergence into A/H.

Adrenal weight changes for the Uinta ground squirrel agreed well with data for the golden mantled ground squirrel presented by McKeever (1964). Adrenal weights did not fluctuate greatly during the activity season, but declined during the fall and were low during the A/H period. The low weights during the A/H period are comensurate with low metabolic activity during A/H and a presumed reduction in activity of the pituitary in secreting tropic hormones. A large weight increase apparently occurs between the end of A/H and emergence. This correlates well with interstitial cell development, androgen synthesis and accessory organ development.

Reproductive Physiology

The pattern of pituitary weight changes reported here are similar to those reported by McKeever (1964) for the golden mantled ground squirrel. The values were fairly constant for the active season,

declined during the preA/H period and were low during A/H. This again indicates a reduced pituitary activty during A/H. The 1974 weights presented here are greater than the 1973 weights. Due to the necessity of freezing the pituitaries for later MAO assay and the longer time the 1973 pituitaries were frozen before being thawed, weighed, and assayed, the 1974 weights are probably a more accurate expression of the squirrel pituitary weights than are the 1973 results. The pituitary weights of A/H squirrels were taken immediately after sacrificing the squirrel and are, therefore, actual fresh weights.

Testicular weight changes, size changes and changes in diameter of the seminiferous tubules were all highly correlated with each other. All three measures of testicular development also followed the reported pattern of testes weight changes in other squirrels (Johnson, Foster and Coco, 1933; McKeever, 1964; McCarley, 1966; Iverson and Turner, 1972). Maximum values occurred at emergence with a rapid decline during May and June. This was followed by a regrowth during the preA/H and A/H periods. These changes most probably indicated a reduction in pituitary gonadotropin release during the emergence and breeding period and an increased release during the preA/H and A/H period to account for the regression and recrudescence of the testes. This hypothesis is supported by the histological preparations of the testes, showing a regression and recrudescence of the germinative layers during the year that corresponded to the testicular development. However, the involvement of some other factor in the regression of the testes is a possibility. Clermont and Morgentaler (1955) reported that

hypophysectomy in male rats reduced only type-A spermatogonia while mitosis and type-B spermatogonia continued to occur and produce spermatocytes. Since in the squirrel, testicular regression is complete, some other factor besides lack of pituitary gonadotropins may play a role in the spring testicular regression.

Testicular steroid secretion, as shown by plasma testosterone levels, did not follow the same pattern as testicular weight, size, seminiferous tubule diameter or spermatogenic activity. Maximum testosterone levels were recorded in the spring at emergence. Thereafter, the levels generally declined as did the testicular development. In contrast to the testicular development, however, no increase in testosterone level was found for the preA/H and A/H periods. The testes experienced a growth and development during this period. That no increase in testosterone production occurred was substantiated by the lack of seminal vesicle development during the same preA/H and A/H periods. The seminal vesicles were highly developed at emergence when testosterone level was highest.

PreA/H regrowth of the spermatogenic elements was confined to proliferation of the spermatogonia without spermatocyte development. FSH has been shown to stimulate mitotic activity in rat spermatogonia (Mills and Means, 1972). LH stimulates androgen production and stimulates spermatogenesis through the effects of androgens. Testosterone stimulates meiotic divisions of spermatocytes (Steinberger, 1971). Proliferation of spermatogonia would, therefore, indicate FSH stimulation while a lack of development of spermatocytes and spermatids

would indicate a lack of LH or testosterone stimulation. The growth and development of the ground squirrel testes, the proliferation of the spermatogonia and the lack of testosterone secretion during the preA/H and A/H periods all suggest that testicular recrudescence is due to stimulation by FSH, but not LH, from the pituitary.

Engel and Frowein (1974) have shown that neonatal Leydig cell insensitivity to human chorionic gonadotropin (HCG) is due to a lack of glucocorticoids in the neonatal circulation. The treatment of neonatal rats with hydrocortisone or adrenocorticotropic hormone (ACTH) and HCG results in increased Leydig cell activity. The data presented here suggested a similar mechanism (i.e., a low plasma level of adrenal corical steroids) may operate during preA/H and A/H periods in preventing the rise in testosterone secretion. Adrenal activity, as shown by adrenal weight changes, decreased during preA/H and was low during A/H (Fig. 2). An increased adrenal activity apparently occurred during the arousal period from A/H (March 13 to April 15). This increase in adrenal activity occurred concomitant with an increase in interstitial cell development, plasma testosterone and accessory organ development. In this respect, the adrenal gland could be partially responsible for the rapid sexual development just prior to emergence of squirrels from their burrows. A low adrenocorticoid secretion during preA/H and A/H may reduce testicular sensitivity to LH stimulation so that little testosterone was produced at this time.

MAO

Testicular MAO activity, graphed as a total for the paired testes, was similar to the pattern of changes in testicular weight, size, seminiferous tubule diameter and spermatogenesis. Activity was high at emergence and declined sharply to a low value in June. The activity then increased significantly during the preA/H and A/H periods when recrudescence of the testes was occurring.

Urry, Frehn and Ellis (1974) have shown that FSH *in vivo* can stimulate MAO in the testes where LH, PRL, or all three combined did not have an effect. FSH also stimulated MAO in tissue culture. Data presented here corroborated the hypothesis that FSH stimulates testicular MAO activity. First, total MAO activity changes were highly correlated ($r = 0.96$) with changes in testicular development that are most certainly under partial control of pituitary gonadotropins. Second, the proliferation of spermatogonia during preA/H and A/H periods, but the lack of development beyond the spermatogonia stage suggests FSH, but no effective LH or testosterone stimulation. Third, the lack of interstitial cell development during preA/H and A/H suggests no effective LH stimulation. Fourth, the lack of an increase in plasma testosterone levels and seminal vesicle weights during preA/H and A/H periods also suggest no effective LH stimulation of the interstitial cell function. The rat and ground squirrel testes may, therefore, both respond to FSH with an increase in testicular MAO activity.

The role of MAO in the testis has been suggested as being one of protection of the testis from the antigonadal effects of endogenous 5-HT

(Urry, Frehn and Ellis, 1974; Urry et al., 1975). Serotonin occurs in the testis (Kormano and Pentilla, 1968) and varies with age of the rat (Zieher et al., 1971). Segal et al. (1975) and Urry (personal communication) reported on urinary changes of 5-HT and 5-HIAA in subfertile men. Mild and grave oligospermic and azoospermic men all had significantly higher 5-HT and 5-HIAA (the MAO metabolite of 5-HT) in their urine when compared to men of normal sperm production ($p < 0.001$) (Segal et al., 1975). Excess 5-HT in the body is excreted without being metabolized while 90 percent of normal 5-HT is metabolized and excreted as 5-HIAA (Segal et al., 1975). The high correlation between human male subfertility and high urinary excretion of 5-HT and 5-HIAA is strong support for a role of MAO in protecting the testes from endogenous 5-HT that could result from psychic stimulation of peripheral tissues.

Exogenous administration of 5-HT has shown that it does collect in various tissues of the testes and epididymis (Hodgen and Gawienowski, 1972; Kormano and Penttila, 1968). Boccabella, Salgado and Alger (1962) found that exogenous 5-HT causes testicular regression. Other evidence for an antigonadal effect of 5-HT is found in the work of Ellis (1972) who demonstrated an *in vitro* block of androgen synthesis and increased conversion of testosterone to androstenedione.

MAO could protect the testis from excesses of endogenous 5-HT. The enzyme has been localized in the testis (Bhagrat, Blaschko and Richter, 1938; Penttila and Kormano, 1968; Urry, Frehn and Ellis, 1974; Urry et al., 1975). The level of activity changes during the development

of the rat, i.e., high at birth then decreasing, peaking again at maturity, then declining with senescence of the testis (Penttila and Kormano, 1968; Ellis et al., 1972). Seasonal changes also occur in seasonal breeders. Urry et al. (1975) found that in house sparrows, testicular MAO activity closely paralleled testicular recrudescence in the spring and regression in late summer and fall. This parallel also occurred in data presented here. Testicular MAO closely paralleled the change in Uinta ground squirrel testicular physiology. Increased levels of MAO activity when the testis is functioning, such as at maturity in the rat or during the breeding season in the sparrow and ground squirrel, could prevent 5-HT from inhibiting spermatogenesis or testosterone synthesis. Conversely, regression of the testes at the end of the breeding season or senescence of the animal may be partially the result of decreasing MAO levels which would allow endogenous 5-HT to inhibit testicular function. Since testicular MAO may be stimulated by FSH, any release of this gonadotropin would increase MAO activity and thereby provide protection from 5-HT while any decrease in gonadotropin secretion would serve to diminish this protection. This mechanism would provide an additional control over testicular development and facilitate the adaptation of ground squirrels to their short activity season.

While a plausible role for MAO in the testes has been developed, no role for MAO in the pituitary gland has yet been found. The anterior pituitary lacks any significant innervation that would require MAO for metabolism of neurotransmitters. Hormone releasing factors are not

thought to be catecholamines or indoleamines. However, since 5-HT is found in the pituitary, MAO may play a role in its metabolism (Piezzi, Larin, and Wurtman, 1970). Highest MAO activity was found in the bovine pars nervosa where highest levels of 5-HT were also found, while low levels of 5-HT occurred in the pars distalis which had only moderate levels of MAO (Piezzi, Larin and Wurtman, 1970). Other authors also report on pituitary MAO levels (Kamberi and Kobayashi, 1970; Urry and Ellis, 1975), but none have discussed its role. Data presented here showed an increase in activity during A/H, a drop after emergence and then an increase during the preA/H period. This pattern would approximate the expected gonadotropin secretion pattern, i.e., an increased secretion for gonadal development prior to emergence; a decline during testicular regression, and an increase during recrudescence of the testes during preA/H. The data, therefore, suggest that changes in MAO activity may play a role in pituitary control of gonadal development.

In the hypothalamus, changes in neurotransmitter release controls the synthesis and release of gonadotropin releasing factors (Kamberi, Mical and Porter, 1970, 1971a, 1971b, 1971c; Muller et al., 1972; Craven and McDonald, 1973; Kordon et al., 1973; Calgaris and Taleisnik, 1974). Changes in MAO activity may play a role in controlling the amounts of neurotransmitter available for release. MAO levels change with stress (Maura et al., 1974) and with the estrous cycle of the rat (Zolovick et al., 1966; Kamberi and Kobayashi, 1970; Holzbauer and Youdin, 1973). Gaziri and Ladosky (1973) reported different activities

in rat hypothalamic MAO between males and females at the time of sexual differentiation of the hypothalamus. Data presented here also demonstrate changes in hypothalamic MAO with changing reproductive condition. Activity was high at emergence, but dropped immediately as did testicular weight and size, seminiferous tubule diameter, and even plasma testosterone. The decrease in hypothalamic MAO may have resulted in increasing 5-HT levels which could then inhibit pituitary gonadotropin release. Hypothalamic MAO then remained at a low level during the post-breeding period. The activity then increased significantly concomitant with testicular recrudescence. Increased MAO activity may cause a decrease in 5-HT levels which would then allow gonadotropin releasing factor secretion. Kamberi and Kobayashi (1970) theorized that a hypothalamic MAO increase on the day of proestrus may be the stimulus needed for the LH surge preceding ovulation. Hypothalamic MAO changes in the Uinta ground squirrel support a role for MAO in the control of gonadotropin releasing factor secretion by the hypothalamus.

Pineal Gland

Pineal NAT activity varies daily in the rat with an activity level 30-50 times greater during the dark-phase than in the light-phase (Klein and Weller, 1970). In the photoperiod experiment reported here, the Uinta ground squirrels did not have a significant difference between the dark (10:00 p.m.) activity level and the light (10:00 a.m.) level. The mean activity in the dark was higher, however, and approached significance ($p < 0.07$) when expressed as counts per 10 minutes per mg

of tissue. This would indicate that there may be a diurnal rhythm in NAT activity in the squirrel. Frehn et al. (1973) were unable to show a difference in HIOMT activity in Uinta ground squirrels between animals kept in an L:D, 14:10 schedule and those kept in constant darkness. However, since the pineal circadian rhythm becomes free-running in constant darkness (Illernova, 1972), one may not find a difference due to the asynchronouy of the biological clocks controlling the pineal gland. Snyder et al. (1965) found that rats kept in complete darkness continue to have 5-HT declines during the normal dark-phase. Klein and Weller (1970) reported that rats in continuous darkness also had peaks in NAT during the time of the normal dark-phase although the rhythms tended to become asynchronous between individuals. Therefore, sacrificing the continuous darkness animals in the morning would probably either find all animals with low HIOMT activities, or, if rhythms are synchronous, animals with greatly varied levels of HIOMT activity. Data presented here seem to be a more accurate analysis of Uinta ground squirrel NAT activity since all squirrels were maintained on the same light-dark schedule and were sacrificed during the light- and dark-phases of the cycle. A pineal rhythm in melatonin production does seem likely for the Uinta ground squirrel.

Pineal NAT activity over the spring-summer seasons and during the A/H period showed a great deal of variation. Although spring-summer seasons for 1973 and 1974 were somewhat different, two common points of interest can be found. In both years the pineal activity was high at spring emergence and declined sharply in 1973, but only gradually

in 1974. The other point of interest is the dramatic drop in activity in mid-March, just before emergence. Reiter (1972a) found in the hamster that placing a male animal in continuous darkness produced testicular regression in a few weeks. If the animal was left in complete darkness, the testes began to recrudesce even though still subjected to constant darkness. This recrudescence was considered to either be due to a decline in sensitivity of the hypothalmo-hypophyseal-gonadal axis to the pineal antigonadal agent or to an exhaustion of the pineal itself (Reiter, 1972a). This recrudescence occurs at about the same time that the A/H period would end in its natural environment. This same phenomenon may occur in the Uinta ground squirrel. The exceedingly low value in mid-March may facilitate the recrudescence of the testes due to a lack of pineal inhibition of gonadotropin release. This would account for the regrowth of the testes while the animal is still in complete darkness in the hibernaculum.

Increased NAT activity at emergence may be due to changes in adrenal function. Sampath and Clarke (1972) have shown that adrenalectomy increased MAO in the vas deferens by 40 percent. Parvez and Parvez (1973a, 1973b) have also shown increased brain, liver, heart and pituitary MAO by adrenalectomy of rats while glucocorticoid treatment reduced MAO in brain and liver. Corticosterone and ACTH administration both increased 5-HT content of the hypothalamus. These results indicate that glucocorticoids inhibit MAO activity. Inhibition of pineal MAO by pargyline or Catron results in a 20 to 40 fold increase in NAT activity (Duguchi and Axelrod, 1972b). In the ground squirrel,

the increased adrenal corticosteroid secretion at emergence over A/H level (Fig. 2) may result in a decrease in pineal MAO activity or a shift from one type of MAO with NE or 5-HT as preferred substrates to another type of MAO that does not metabolize NE or 5-HT as readily. The result would be an increase in NE and 5-HT levels in the pineal and, therefore, an enhanced NAT activity (Klein, Berg and Weller, 1970).

The high activity at emergence may also serve to hasten the regression of the testes. High pineal activity causes regression of hamster testes as shown by exposure of active adult males to short daily photoperiods (Reiter, 1972a). Melatonin inhibited testicular development in Djungarian hamsters when exposed to long photoperiods (Hoffmann, 1973), but prevented testicular involution caused by short photoperiod (Hoffmann, 1974; Reiter et al., 1974). Melatonin also caused testicular regression in rats (Debeljuk et al., 1971). Melatonin injection caused an increase in 5-HT content in the brain (Anton-Tay et al., 1968) as well as an increase in NE and dopamine (Wendel, Waterbury and Pearce, 1974). These catecholamine and indoleamine increases may be the result of decreased MAO activity. Urry and Ellis (1975) demonstrated that melatonin decreased <u>in vitro</u> hypothalamic MAO activity while pinealectomy increased MAO activity slightly. High pineal activity at emergence, possibly acting through melatonin secretion, could inhibit secretion of gonadotropin-releasing factors by decreasing hypothalamic MAO and allowing hypothalamic 5-HT to block gonadotropin release, thus causing testicular regression. In 1974, hypothalamic MAO did decrease sharply after emergence while the pineal

activity remained moderately high suggesting that perhaps the pineal antigonadal agent did contribute to a fall in hypothalamic MAO activity. During the 1974 A/H period when pineal activity was low, hypothalamic MAO activity increased significantly.

SUMMARY

1. Male Uinta ground squirrel activity and physiological changes over the two spring-summer seasons and during the A/H period agreed closely with data collected by other researchers for this genus and species.

2. Squirrel body weights for years with early average emergence were higher than body weights for years with late average emergence.

3. Seasonal changes in testicular development were similar to data reported for this species and other ground squirrels in that development was maximum at emergence; the testes regressed just before or shortly after emergence then recrudesced during the preA/H and A/H periods. Maximum testicular growth appeared to occur between mid-March and emergence.

4. Testicular weight, length, and width, and seminiferous tubule diameter were highly correlated to each other.

5. Histological preparations showed that development of the germinal epithelium followed testicular development closely: maximum development at emergence, rapid regression followed by recrudescence (as shown by proliferation of spermatogonia).

6. Plasma testosterone levels and seminal vesicle weights did not follow the same pattern found in testicular development. Testosterone levels were highest at emergence and decreased rapidly with testicular regression. In contrast to testicular development, however,

no increase was seen during the preA/H period in testosterone levels or seminal vesicle weight.

7. Testicular MAO expressed on a per mg of tissue basis followed changes in plasma testosterone levels and seminal vesicle weight. Testicular MAO expressed as a total activity for the paired testes followed the pattern of testicular development. Total testicular MAO changes constituted a more accurate representation of testicular MAO activity.

8. Data presented here substantiate the hypothesis that FSH stimulates testicular MAO activity in that: a) total MAO activity changes were highly correlated with testicular development; b) proliferation of spermatogonia during preA/H and A/H periods but lack of development of spermatocytes of spermatids suggests FSH stimulation, but no effective LH or testosterone stimulation; c) lack of interstitial cell development during preA/H and A/H periods also suggest no effective LH stimulation; and d) no increase in plasma testosterone or seminal vesicle weight also suggest no effective LH stimulation during this same period.

9. An increase in MAO activity with the development of testicular function could protect the testes from the antigonadal effects of 5-HT while decreased MAO activity could facilitate testicular regression after the breeding season.

10. Pituitary MAO activity followed the expected gonadotropin secretion pattern by the pituitary during the activity season.

11. Hypothalamic MAO activity followed the expected gonadotropin secretion pattern and may have operated through different levels of 5-HT in the hypothalamus.

12. Diurnal variations in pineal NAT activity approached significance ($p < 0.07$) in that 10:00 p.m. values were greater than 10:00 a.m. values.

13. A significant drop in pineal NAT activity in March just before arousal-emergence may facilitate testicular development at this time. High activity at emergence may contribute to testicular regression during the breeding season.

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APPENDIX

Common and Scientific Names of Species Used in the Manuscript

Uinta ground squirrel	*Spermophilus armatus*
Golden hamster	*Mesocricetus auratus*
Rat (white, laboratory)	*Rattus rattus*
Franklin ground squirrel	*Spermophilus franklini*
Thirteen-lined ground squirrel	*Spermophilus tridecemlineatus*
Richardson ground squirrel	*Spermophilus richardsoni*
Golden mantled ground squirrel	*Spermophilus lateralis*
Mouse (white, laboratory)	*Mus musculus*
Human (man, men)	*Homo sapiens*
Dog	*Canis familiaris*
European suslik	*Citellus citellus*
House sparrow	*Passer domesticus*
Horse	*Equus caballus*
Sheep	*Ovis aries*
Goat	*Capra hircus*
Mink	*Mustela vison*
American chemeleon	*Anolis carolinensis*
Djungarian hamster	*Phodopus sungorus*
Whitefooted mouse	*Peromyscus leucopus*
Cattle (cow, steer)	*Bos taurus*

VITA

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